Opportunities and strategies for introducing pharmacogenetics into early drug development

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Following the publication of the first draft of the human genome, this is a good time to re-analyse the potential contribution of genomics to drug development. Pharma, biotech and academia are already queuing up to deliver novel data impinging on every aspect of medicine and we can foresee a five-year scenario in which every new drug with a known mode of action will have a target gene sequence in the public domain. As such, current development strategies must ultimately be capable of anticipating and addressing genetic issues. This article attempts to position recent developments in genomics from an industrial perspective.

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▼ The first draft of the human genome (http://www.ncbi.nlm.nih.gov/genome/guide/) has now been published and we have made tremendous advances in mapping polymorphism within the human genome (http://snp.cshl.org/news/snp_hgp_collab.html). Now thus seems to be an excellent time to look at the potential contribution of genomics to early drug development within the pharmaceutical industry. Although it is too early to expect to see direct value from this heavy investment in genomics, the practice of commercial drug development will inevitably be altered. Every novel drug with a known mode of action will soon have information on target gene sequences and polymorphism in the public domain, and customers, regulators and competitors will make use of this.

Successful early development strategies must anticipate and determine genetic issues, and must be capable of definitively resolving them. Consequently, clinical genetics within pharmaceutical development should be seen as a novel, high-profile and rapidly growing activity[1]. For several years, this type of work has been undertaken within pharmaceutical companies as part of an attempt to drive a transition from classical target acquisition to the use of drug targets ascertained from genomic research[2]. However, clinical genetics must now be envisaged as not only an isolated discovery strategy but also a devolved and integrated activity capable of reaching across research and development boundaries. It must be directly geared towards achieving the goal of faster development of more drugs with greater clinical efficacy[3], while simultaneously ensuring inherently better market definition for such drugs.

Integrated commercial pharmacogenetics is aimed at maximizing the value of pharmaceutical research and development by increasing the capacity for discovery of novel drug targets, the selectivity of drug target validation, the clinical effectiveness of drugs and the clinical safety of drugs. Clinical genetics offers the industry a unique opportunity to increase the value of drug portfolios in terms of healthcare-related effectiveness. It is a widespread contention that this integrated process must inevitably begin within early development, with the implementation of simple pharmacogenetic trials exploiting a very limited range of well-proved genetic analyses. Such analyses are likely to be based around candidate genes already very strongly identified with pharmacokinetic parameters or the disease process.

As pharmacogenetics evolves, such simple intuitive strategies for genetic analysis will be increasingly replaced by a comprehensive, systematic and data-driven model in which data from deliberate pharmacogenetic analyses within early trials are incorporated. Such deliberate strategies will draw on a wide range

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Box 1. Genetic variation and the cytochrome P450s

The hepatic haem–thiolate proteins referred to as cytochrome P450s form a superfamily of membrane-bound enzymes that play a key role in the primary metabolism of both xenobiotic and endogenous compounds, such as drugs and hormones[a]. To be enzymatically active, they require the presence of a second membrane-bound protein, NADPH P450 reductase, which transfers electrons from NADPH to the P450 (Ref. b). The P450 proteins are categorized into families and subfamilies according to sequence similarities. To date, at least 53 human *CYP* genes and 24 pseudogenes have been recognized (http://www.imm.ki.se/CYPalleles/).

CYP enzymatic activity is known to be highly variable at both inter- and intra-individual levels. This can be at least partially explained through essential variations in catalytic rate. This is thought to be due to genetic polymorphism affecting the amino acid structure of the CYP enzyme. The best-known examples of such mutations include *CYP2C19*2* and *CYP2D6 del506*, in both of which a single base-pair deletion gives rise to a frameshifted and prematurely truncated version of the CYP peptide with markedly reduced catalytic activity[c,d]. Enhanced gene transcription might also play a role in underpinning the observed variability in CYP activity, in that many CYP enzymes are 'inducible' by xenobiotics. Such enhancement of *CYP* gene expression is thought to rely on the xenobiotics binding to and activating nuclear migratory receptors (xenosensors), such as PXR/SXR, which can in turn bind to and enhance transcription from specific *CYP* gene promoters[e].

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of potentially informative genetic assays, deriving data from sources as diverse as automated literature searches, expression array profiling (http://www.gene-chips.com/) and the experimental use of single-nucleotide polymorphism (SNP) maps of the human genome[4] to elucidate mechanisms of disease.

To date, the industry has demonstrated a strong interest in building the utility of such data sources in preparation for such comprehensive approaches to pharmacogenetics. In the case of SNP maps, this enthusiasm has extended to a substantially funded collaborative venture with the public sector aimed at delivering a universally available high-density map of single nucleotide polymorphism across the human genome. However, the early completion of the sequencing phase of the Human Genome Project and the subsequent reassignment of sequencing capacity to SNP discovery might already have superseded the direct requirement for such independent efforts before their completion.

Rationale for pharmacogenetics

Inter-individual variability in response to a fixed dose of a therapeutic agent is well described. For any single disease, this might be attributed to the variability of pharmacokinetic parameters such as drug absorption, metabolism and excretion, and of pharmacodynamic parameters such as the sensitivity of pharmacodynamic systems. Alternatively, variability in the response might be attributable to a seemingly homogeneous disease phenotype being in fact underpinned by etiologically distinct disease subtypes. All these variable traits can, of course, carry a significant genetic contribution[5].

'Classical' pharmacogenetics has historically focused on attempts to analyse variation in pharmacokinetic parameters, with the eventual aim of predicting the genetic contribution to inter-individual variations. This has predominantly involved the examination and characterization of common polymorphism in enzymes of hepatic phase I (oxidoreductive) and, to a lesser extent, phase II (conjugatory) metabolism[6]. In the near future, we confidently expect this situation to undergo rapid evolution, in alignment with significant developments in our understanding of genomic etiology. This will extend the scope of genetic analyses of pharmacogenetic determinants beyond those genes directly linked to drug metabolism and related parameters. This conceptual division of pharmacogenetic factors into 'pharmacokinetic' and 'pharmacodynamic' determinants is an important one and can support a methodological analysis of the potential use of clinical genetics across early development.

Pharmacokinetics

Traditional, pharmacokinetics-based pharmacogenetics [e.g. the well-described genetic polymorphism seen with several hepatic haem–thiolate proteins (Box 1) or UDP glucuronosyltransferases (Box 2)] can be used to help ensure that trial cohorts are representative of, or informative regarding, the host population[7,8]. Such strategies extend but do not challenge the current models for drug development

Box 2. Genetic variation and the UDP glucuronosyltransferases

The reticular uridine 5′-diphosphate glucuronosyltransferases (UDPGTs) are responsible for catalysing the phase II hepatic glucuronidation of small lipophilic agents[a]. This completes the aqueous solubilization of endogenous metabolites and xenobiotics (>350 substrates have already been discovered). To date, at least 15 human UDPGTs have been identified and characterized at the genetic level (http://www.unisa.edu.au/pharm_medsci/Gluc_trans/). Of principal pharmacogenetic note, genetic studies directed at understanding the role of the *UGT1A* gene product in bilirubin glucuronidation have uncovered coding sequence and promoter polymorphisms associated with Crigler–Najjar's and Gilbert's syndromes, respectively[a]. Similar functional polymorphisms should be anticipated in all UDPGTs with marked pharmacogenetic potential[b].

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Box 3. The pharmacogenetics of thiopurine metabolism

Thiopurine methyltransferase (TPMT) catalyses the *S*-methylation of thiopurine drugs. Genetic polymorphism of TPMT plays an important role in underpinning variations in the pharmacokinetics of the antileukaemic and immunosuppressive medications mercaptopurine, thioguanine and azathioprine[a]. Several allelic variants of TPMT reproducibly associating with low enzymatic activity have been reported[b]. The most common are two nucleotide substitutions (460G→A and 719A→G) that produce the amino acid changes 154Ala→Thr and 240Tyr→Cys, respectively. Either mutation can lead to a reduction in catalytic activity, whereas the haplotypic presence of both mutations leads to complete loss of enzymatic activity[c]. Genetically determined TPMT activity might be an important regulator of the cytotoxic effect of mercaptopurine, which might translate into therapeutic effects[d,e].

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and, as such, have already found acceptance and employment in the pharmaceutical industry, clinical research organizations and academic groups. Assays for hepatic haem–thiolate-enzyme polymorphisms (CYP2D6, CYP2C9, CYP2C19) that comply with Good Laboratory Practice regulations are readily commercially available. This means that it is unlikely that any compound currently under development with a significant dependence on polymorphic CYP enzymes for its metabolism would escape pharmacogenetic investigation, at least in a precautionary fashion.

This pharmacokinetics-led investigative approach is particularly useful when such functional polymorphism has a demonstrably diverse racial distribution, as is the case for CYP2C19 metabolizer status in European and Asian populations (homozygous or compound heterozygous poor metabolizers composing up to 5% and 20% of these populations, respectively)[9]. This ability to ensure adequate representation can enable the most appropriate testing of any drug in terms of pharmacokinetic and safety considerations, and can also result in greater sensitivity and specificity of response detection. Exceptionally, although it must become more common within the next two to three years, this concept could be extended to accommodate active, deliberate subject selection according to their alleles related to metabolizer status. This approach allows phase I and II trials to to deliver information on rare but pivotal subgroups of the target population. Such genetic information might prove to be an important factor in facilitating transnational bridging of clinical trial data[10] and can assist subsequent population modelling of pharmacokinetics and dynamics.

Knowledge of metabolic polymorphism can also help to establish rational dosing regimens on a firm scientific basis[11]. Early experience from the field of chemotherapy shows how benefits can be derived from using the genetic analysis of metabolic enzymes to predict individual therapeutic requirements by, for example, determining chemotherapy doses according to polymorphism in thiopurine metabolism (Box 3). Significant examples of reported 'pharmacokinetic' genetic polymorphism are given in Table 1.

Table 1. Reports of pharmacokinetic polymorphism

	Enzyme polymorphism	Affected drugs
Oxido-reductive phase I enzymes	ADH	Ifosamide, cyclophosphamide
	CYP 450s	Antiarythmics, antihypertensives, neuroleptics etc.
	DPD	5-Fluorouracil
	G6PD	Primaquine etc.
	Esterases	Succinyl choline
Conjugating phase II enzymes	GST	Multi drug resistance
	NAT	Isoniazid, sulfonamides
	TPMT	6-Mercaptopurine, azathioprine
	UDPGTs	NSAIDs

Pharmacodynamics

Modern pharmacogenetics has moved to encompass not only the analysis of pharmacokinetics but also elements relating to inter-individual variability in pharmacodynamic systems and disease pathology. Of particular relevance to the use of genetic analyses in the early stages of drug development is the ability to demonstrate association between target variation and pathology. This enhances confidence in the existence of an etiological role for a putative drug target before the drug is used in humans (a process sometimes termed 'target validation')[12]. In addition, hypothesis-generating analyses of genetic sub-cohorts from phases I and IIa might inform therapeutic 'proof of concept' planning in later phase II studies. As previously noted for pharmacokinetic studies, selecting subjects for phase IIa trials according to alleles demonstrably associating with a particular disease etiology can help to elucidate drug effects on rarer phenocopies of common disease in small studies. This can help to provide early information on rare alleles and genotypes that are insufficiently common for reliable passive representation in small cohorts that are representative of the wider population. It can thus provide safety data that, under normal circumstances, might only be picked up by post-marketing surveillance.

Even though, as indicated, the genetics of pharmacokinetic differences is well established in current usage, the extension of the concept to pharmacodynamic considerations is much harder to demonstrate. Certainly, keynote presentations have highlighted the capacities for such studies in several large pharma companies but, as yet, such data as might exist remain either rudimentary or confidential. The next five years might see the emergence of several examples of genetically targeted therapies being offered for regulatory review. Their success or otherwise at this hurdle, and subsequently in the market, will set the scene for the eventual adoption of pharmacodynamics-based pharmacogenetics within the industry. Certainly, the therapeutic and commercial success of Herceptin™, with its reliance on an indirect assay of gene expression to inform prescription, bodes well. Significant examples of reported pharmacodynamic genetic polymorphism are given in Table 2.

Table 2. Reports of pharmacodynamic polymorphism

Target, pathway or disease related polymorphism	Affected drugs
HTR2, HTR6, DRD3	Clozapine
CETP, LPL, LDLR	HMGCoA RIs
APOE	Tacrine
ALOX5	5 Lipoxygenase inhibitors
B2AR	B2 Adrenergic agonists
KCNQ1	Cisapride
CCR5	Anti-retrovirals

Ethical and societal considerations in commercial pharmacogenetics

There is a high degree of concern within professional and lay groups that the potential ethical, personal and societal implications of undertaking exploratory clinical genetic analysis should be explicitly limited in extent[13,14]. Although such concerns do apply to 'academic' uses of genetics (http://www.wellcome.ac.uk/en/images/DNAsamplecollection_background_2261.pdf), they often seem to be particularly related to the commercial exploitation of genetic resources[15]. Against this background, a pragmatic and precautionary attitude to the introduction of genetic research into late-phase pharmaceutical trials has resulted in the widespread adoption of non-identified sample banks, in which specific steps (often termed sample anonymization) have been taken to remove evidence of the identity of the source subject from the data accompanying clinical samples (i.e. deletion of name, trial ID number, date of birth etc.). These procedures are often coupled to policies of non-disclosure of trial-derived genetic information other

than in aggregate and non-identified form. Such techniques of 'genetic exceptionalism' provide researchers with the reassurance of using a disconnected data set, but this is purchased at the expense of the loss of individual, traceable analysis[16]. In this respect, the pharmaceutical industry would seem to be leading best ethical practice and to have been amongst the first 'genetics users' deliberately to address issues of consent and privacy implicit in DNA banking[17].

Parenthetically, it should be noted that similar degrees of data privacy to that stemming from sample anonymization processes can also be provided solely through techniques of identity encryption[18,19]. In such methods, the identity of samples is not deleted but is instead stored securely, such that it is only accessible to a very limited set of individuals with specific responsibility to protect and maintain the privacy of sample donors. A specific variant on this theme is 'one way' encryption. This is a dynamic process, without the necessity to limit analysable data sets, through which the key allowing data entry can be given widespread availability without compromising the security of the decryption process. This technique has specific advantages for control of data privacy and, as such, has been adopted by a significant minority of research groups. Limitations on the aggregate analysis remain, of course, and some commentators have expressed concern at the extent of security achieved through sole reliance on encryption.

However, in addition to hypothesis-generating genetic research, there is also a requirement within drug development to study certain well-recognized polymorphisms, with established phenotypic correlates. This requirement is particularly prevalent in early development trials. Historically, such functional genetic variants have often been revealed at the phenotypic level but, with new technology, they can now be more easily demonstrated at the genetic level[20]. To this category belong the polymorphisms at the hepatic haem–thiolate-metabolizing enzymes already noted (Box 1). Future developments in genetic research and knowledge will undoubtedly see the number of such clinically validated genetic polymorphisms growing significantly. In these instances, genotyping might eventually need to be an integral part of the development and labelling strategy, and can be seen to be similar to including surrogate markers of disease, HIV status, smoking habits or other covariates needed for interpreting any eventual results. As such, the currently widespread approach of ensuring 'exceptional' levels of privacy for genetic information (such as anonymization or encryption) will need to be modified in light of the inherent clinical value of individual and traceable data within an externally regulated development process.

Potential regulatory acceptability of pharmacogenetics

The acceptance of the likely future importance of pharmacogenetic approaches to drug development by regulatory authorities is evident, and the future impact of pharmacogenetics on requirements for registration of novel chemical entities should not be underestimated. Such consideration might even eventually extend to second-level institutes of clinical governance. The following areas relating to the use clinical genetics within drug development have already received mention from the US Food and Drug Administration (http://www.fda.gov/default.htm): understanding trans-racial metabolic heterogeneity as it relates to pharmacokinetics and pharmacodynamics; and predicting drug safety and efficacy against the background of inter-individual heterogeneity of drug metabolism. The reports to date reflect a bias towards applications of pharmacogenetics relating to pharmacokinetic variability. As such, there are few available data to inform any prediction of future regulatory response to the presentation of more etiological genetic data, in support of registration of a diagnostic or therapeutic package.

Conclusion

Important, far-reaching claims have been made about the impact of the genomics on medicine during the next decade. Although these probably need to be tempered with some realism, current information and practice will undoubtedly result in clinical genetics affecting drug development. The brunt of such changes will inevitably be borne in early development. As processes develop to explore the scientific possibilities inherent in genetic data, the usefulness or otherwise for subsequent mid- to late-phase development strategies will become apparent. Success in this area of early development will come from the use of novel technology in an environment of traditional development competencies. Introducing pharmacogenetics into early development will call for proactive planning based on sound scientific processes and systematic reviews of relevant issues.

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