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Bioinformatics and Drug Safety

Examining how genetic analysis can be useful in designing drug regimens

by John Hammond

Bioinformatics and Drug Safety
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Executive summary

Establishing the safest and most efficient drug regimen for a patient can be a trial-and-error process that can, in some cases, place patients at risk. Variability in response to certain drugs between different patients has major implications for the pharmaceutical and healthcare industries.

The ability to determine the correct drug and dosage for therapy at the outset would contribute significantly to drug safety. It would also lead to a more economical use of drugs, with potential savings for healthcare providers.

It is becoming clear that variability in drug response may be inherited. With the application of genetic analysis, DNA analytical techniques and pharmacokinetics, it has become possible to identify the genes, and the proteins which they encode, that are involved in drug responses.

This Expert Review, *Bioinformatics and Drug Safety*, looks at how to identify the genes involved in drug response, characterise the variants and devise a convenient means of identifying variants in a patient. It shows how genetic analysis can be a useful tool in designing the drug regimen for the best risk/benefit, and makes a case for genotype testing prior to prescription, and for genotype determination as a part of the clinical trial process.

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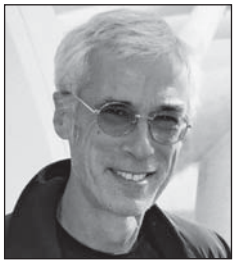

The past decade has seen a rapid accumulation of data on the genetic content of the human cell. At the same time, fast and economic techniques for DNA analysis have been developed. As a consequence, we are now at a stage where genotype–drug interactions can be investigated relatively easily and the information used to reduce risk in drug therapy.

The proportion of individuals in the population who are sensitive to a drug by genotype, however, is small. This makes statistically significant data on drug sensitivity by genotype difficult to acquire, and means that validating the relationship is a challenge.

Yet the challenge is not insurmountable. Approaches exist through which it is possible to identify genes that regulate the hitherto unknown processes that modify drug response. By comparing the genetic profile of individuals who respond differently to a given drug with those of normal responders, identification becomes possible.

Use of genetic data and techniques also has the potential to assist the development of drugs against new and better-defined targets than those produced in the past, as well as influence how they are tested. Nevertheless, although knowledge of genetic influences on drug response is emerging as an important development for drug safety, there are some natural limits to its application. There is little sense in applying genotype testing to drugs for which there is already an adequate means of predicting dose outcome. Nor is it useful to determine genotype where there is no significant difference in drug response *in vivo*, even when alleles causing abnormal drug metabolism *in vitro* are present. Despite this, there are cases where genotyping has obvious benefits for safety.

Bioinformatics and Drug Safety assesses these benefits, outlining where the best gains for drug safety can be made and how genotyping can help form part of the solution.

John Hammond
February 2006

About the author

John Hammond graduated in Biochemistry and Soil Science from the University of Wales, following this with research for an MSc in the same subject. He was later awarded a DPhil in fungal biochemistry from the University of Sussex. He worked as a government research scientist at the Glasshouse Crops Research Institute, and then at Rothamsted Experimental Station for 17 years, working on fungal and plant biochemistry, molecular biology and genetic manipulation, and authoring over 40 research papers. He has also held visiting research fellowships at the University of California, King's College, London and the University of Surrey.

After leaving full-time research he was appointed senior lecturer, and later reader, in molecular genetics at the North East Surrey College of Technology. In these positions he developed and taught courses on molecular genetics, biotechnology and DNA technology for undergraduate and postgraduate students. As well as continuing with part-time lecturing in medical and molecular genetics at the college, he now writes on molecular and genetic aspects of medicine, and constructs websites and online learning materials for healthcare companies, as principal of Bioupdates Consultants. He is a Member of the Institute of Biology and a Chartered Biologist.

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Bioinformatics and Drug Safety

Drug response variability

The wild card in drug safety is the considerable variation in response to certain drugs between different patients. While a specific dose of a drug may have the desired effect in one patient, another may experience severe side effects, or no effect whatsoever. This means that establishment of the safest and most efficient drug regimen for a patient can be a trial-and-error process that in some cases can place the patient at risk.

This variability in response has major implications for the pharmaceutical and healthcare sectors. For the pharmaceutical sector it may mean that drugs fail in clinical trials because of toxic effects on a small, but undefined, proportion of patients. Or, once approved, the harmful effects of a drug in use may lead to bad publicity, lawsuits and drug withdrawal, because the small group of individuals for whom the drug is toxic was not included in the random selection for clinical trials.

For healthcare delivery, known variability in drug response means that careful monitoring of drug effect, through laboratory tests of various types, needs to be implemented to arrive at the most beneficial dose for the individual patient. This may involve repeat testing over a period of days or weeks and has major cost implications.

Known variability in drug response means that careful monitoring of drug effect needs to be implemented to arrive at the most beneficial dose for the individual patient; this has major cost implications

The ability to determine the correct drug and dosage for therapy at the outset would therefore contribute significantly to drug safety. It would also lead to a more economical use of drugs, with potential savings for healthcare providers.

The implications of interpatient variability in drug response are well illustrated in cancer chemotherapy. Here, ineffective undertreatment is undesirable since it allows continued tumour growth, and overtreatment causes toxic effects. In chemotherapy, drug dosage is normally calculated as a function of body surface area, assuming a uniform response among patients. This is not always adequate, however. For example, the drug mercaptopurine is used as chemotherapy for childhood acute lymphoblastic leukaemia (ALL), as well as in the treatment of autoimmune diseases and transplant recipients. As cytotoxic agents, the mercaptopurines have a narrow window of effective dose; levels above this window can lead to serious drug-induced toxicity. If a standard starting dose for childhood ALL based on body surface area is given, the calculated exposure to the drug based on plasma concentration and time can vary by as much as 70-fold between patients. High levels of drug exposure can lead to potentially fatal haematopoietic toxicity, and careful monitoring of the effects of exposure through the degree of suppression of the neutrophil count is required.[1]

Genetic influence on drug response

Over the past two decades it has become increasingly clear that variability in drug response may be inherited. With the application of genetic analysis, DNA analytical techniques and pharmacokinetics, it has become possible to identify the genes, and the proteins which they encode, that are involved in drug responses. In those cases in which there is an inherited variation in drug response, it is reasonable to assume that this is due to small variations in the gene, leading to variations in the activity of the protein that it encodes. Characterisation of the gene variants (or alleles) and matching of variants with drug response therefore lays the groundwork for the development of testing to determine which variant a patient possesses and therefore what their response to a specific drug is likely to be (Table 1).

Variability in drug response may be inherited

When this understanding is applied to the case of variability in response to mercaptopurine, the possibility for benefits in drug usage can be seen. A number of investigations have shown that the variability in response to mercaptopurine is related to the enzyme

Drugs	Gene
Erythromycin Isoniazid Rifampicin	CYP1A2
Fluoxetine Ibuprofen Isoniazid Warfarin	CYP2C9
Fluoxetine Rifampin Imipramine	CYP2C18
Ibuprofen Naproxen Isoniazid	UGT2
Isoniazid Procainamide	NAT2
Mercaptopurine Azathioprine Daunorubicin	TPMT
Allopurinol	HLA-B
Abacavir	HLA-B

Table 1. Some examples of drugs that are known to vary in effect as a result of genetic variation. The genes responsible are shown. Note that some drugs are influenced by more than one gene.

thiopurine methyltransferase (TPMT) and the gene that encodes it. The enzyme inactivates the drug, so that the effective dose is reduced *in vivo*. However, 6% of alleles of the TPMT gene in the population cause very low enzyme activity, leaving a concentration of active drug in the body that is high enough to cause toxicity. Although interpatient variability can be compensated for with careful monitoring of neutrophil level and dose adjustment, prediction of an individually appropriate starting dose is a safer option. For patients with a deficiency in TPMT, as measured by enzyme activity or DNA-based allelic analysis, a safe dose is 10–15% of that for those with normal TPMT activity.[1] Thus, previewing the outcome through genetic analysis can be a useful tool in designing the drug regimen for the best risk/benefit.

Previewing the outcome through genetic analysis can be a useful tool in designing the drug regimen for the best risk/benefit

The mechanism of inheritance

If we are to understand fully how different gene alleles affect drug response, and how genetic analysis can inform drug therapy that is safe and effective for all individuals, we need to understand how genes are inherited.

The cells in our bodies contain two copies of each of the 22 non-sex chromosomes (autosomes) and the two sex chromosomes (two X chromosomes in females; one X and one Y chromosome in males). We inherit one copy of each autosome and one sex chromosome from each of our parents (Fig. 1), and therefore have two copies of most genes. Genes carry the information required to accurately assemble a protein in the quantities required to execute effectively its appropriate function in the cell. The information is carried in the form of a code consisting of various combinations of four components (the nucleotides or bases: adenine [A], guanine [G], cytosine [C] and thymidine [T]) of the linear DNA polymer. The base sequences that determine the structure/function of a protein conform to the 'genetic code'.

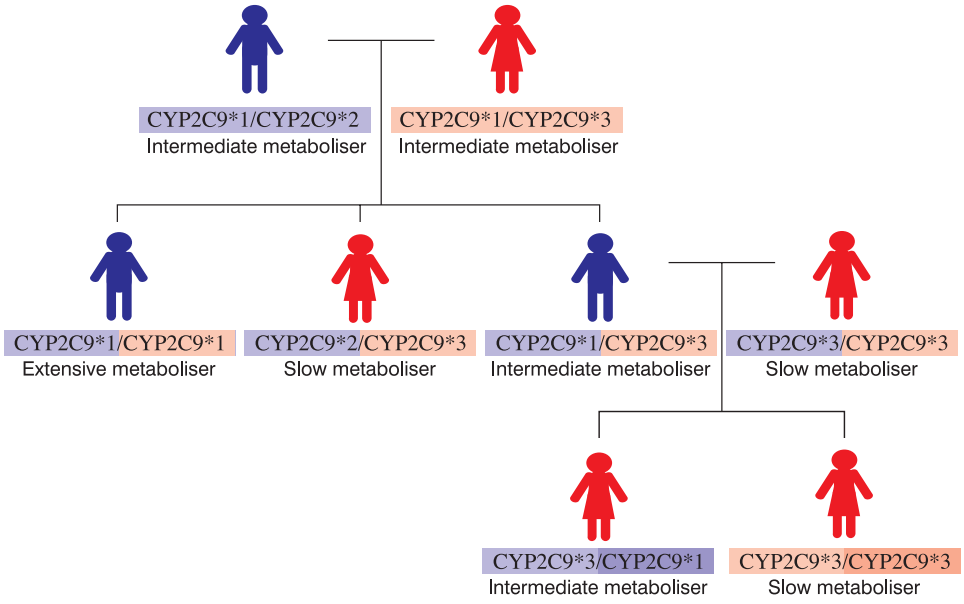

Fig. 1. An individual inherits two copies of each gene, one from each parent. The combination of alleles thus inherited determines the characteristic displayed. In this example three alleles of the cytochrome P450 gene CYP2C9 (CYP2C9*1, *2 and *3) are shown, illustrating the way in which they determine how drugs are metabolised. In fact, the normal allele CYP2C9*1 is predominant in the population.

Because the code is formed from chemical entities it is subject to small changes that can be triggered by physical (e.g. radiation) or chemical (e.g. tar oils) agents. These changes may lead to a permanent change (a mutation) in the base sequence of coding or control regions of a gene. Mutations may cause significant, minor or no change in the function or quantity of the protein. If the mutations occur in those cells that contribute to the formation of sperm or egg (germline cells), they will be passed on to any offspring.

Consequently, after a period of time we will find a number of different alleles of a particular gene if we look at a range of individuals across a population. The alleles may differ by as little as one base in a sequence of several thousand. It is quite possible that the two alleles of a gene that we have inherited (one from each parent) will be different. The likelihood of this happening will depend on the frequency of each allele in the population at large from which our parents originated. If one of the alleles produces a protein that has reduced or no function this lack may be overcome by the effective protein produced by the 'normal' (or 'wild-type') allele of the other copy of the gene. This could be seen as a sort of 'failsafe' mechanism. The term homozygous is used to describe the allele complement (or genotype) of an individual when both alleles of the gene are identical; the term heterozygous is used when the individual possesses two different alleles of the gene.

Because of the failsafe effect, loss of protein function caused by a specific allele may not be obvious in an individual heterozygous for that gene, if the other allele is the wild type. In the case of TPMT, however, this is not true. Patients who are heterozygous with one wild-type and one low-activity allele have lower than normal TPMT enzyme activity. As a result of this, TPMT heterozygous patients can only tolerate 15% lower doses of mercaptopurine than patients homozygous for the wild-type allele.

TPMT heterozygous patients can only tolerate 15% lower doses of mercaptopurine than patients homozygous for the wild-type allele

Using genotype to predict safe drug dosage

The mercaptopurine/TPMT example shows that knowledge of the genetic profile of a prospective patient can lead to safer and more effective drug treatment. So, how widely can this approach be applied? The effect of drugs on the body is subject to the activity of a range of different types of proteins and their functions (Fig. 2). For example, an immediate response to a drug may rely on the stimulation of a specific receptor by that drug (the drug target), which mimics the action of a natural messenger such as a hormone. However, the drug is likely to be subject to other processes in the body that remove its activity, as we have seen with mercaptopurine and TPMT. Equally, some drugs are inactive as applied and are activated in the body by specific enzymes. Thus, for every drug type there is a range of proteins (and their genes) that can affect the level of response. If alleles of any of these genes exist that produce proteins of lower or higher than normal activity, there will be implications for the response to drug dosage. So how are we to identify the genes involved in drug response, characterise the variants and then devise a convenient means of identifying the variants in a patient?

In order to get valid results in pharmacogenetic research, studies that include large numbers of participants with relatively few allelic variants are required. This often

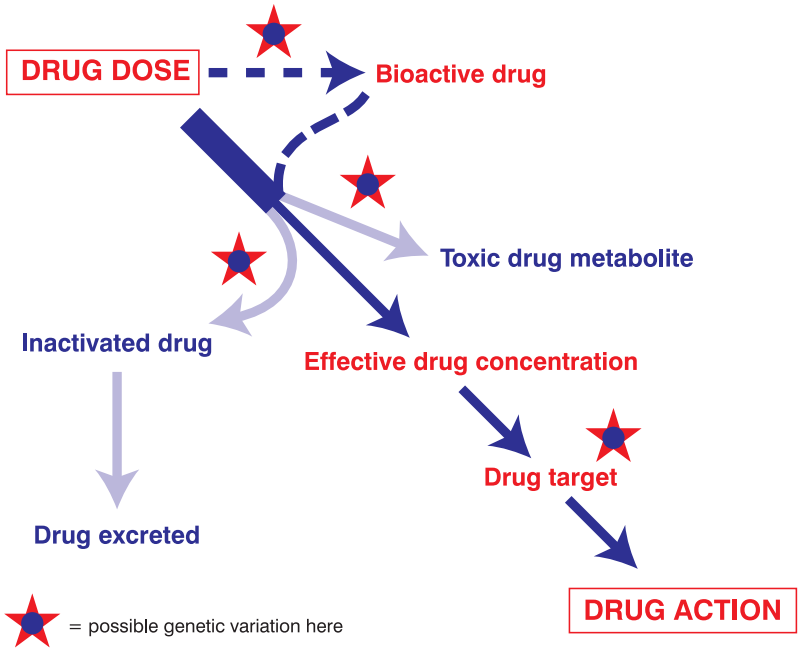

Fig. 2. The effect of a drug is influenced by the concentration of bioactive drug at the target (e.g. a receptor) and the ability of the target to react to the drug. Effective drug concentration and receptivity of the target may be reduced or increased by processes regulated by proteins and be subject to genetic variation.

Drug	Poor metaboliser characteristics
Warfarin	Bleeding – lower dose
Phenytoin	Ataxia – lower dose
Ibuprofen	Gastrointestinal bleeding – lower dose
Tolbutamide	Hypoglycaemia – lower dose
Cyclophosphamide	Reduced effect – higher dose?
Fluoxetine	High sensitivity – lower dose

Table 2. Some drugs that are affected by cytochrome P450 CYP2C9 genotype. The effect of the drug on poor metabolisers (individuals with no wild-type allele of the gene) and palliative action are shown.

means looking at drugs in widespread use against common conditions. Asthma is a good example. Asthma is commonly treated with beta$_2$-agonist drugs, which act as bronchodilators, relieving the condition by relaxing the muscles around the lungs. These drugs are generally considered to be the best treatment for patients with mild, intermittent asthma.

Beta$_2$-agonist drugs act on the beta$_2$-adrenergic receptor in a manner similar to that of the hormones adrenaline and noradrenaline, but are longer lasting and act more directly on the airways to the lungs. Salbutamol, salmeterol and albuterol are examples of beta$_2$-agonists and are widely used in asthma therapy. However, their effect varies between patients. There is evidence that some of this variability is inherited. The beta$_2$-adrenergic receptor has been the subject of extensive research, and the gene and protein sequences are known. Several alleles have been found.

A recent clinical study compared the effect of albuterol on patients carrying two alleles of the beta$_2$-adrenergic receptor gene.[2] The alleles differ in the amino acid at position 16 in the beta$_2$-adrenergic receptor protein. The most common allele produces a receptor with the amino acid arginine in this position; in the second allele the amino acid is glycine. Previous work had shown that the homozygous glycine/glycine genotype is more prevalent in patients with nocturnal asthma, and that there were possible differences in response to albuterol between arginine/arginine and glycine/glycine genotypes. The arginine/arginine genotype is found in around 16% of asthma sufferers, although there are some differences between population groups. The heterozygous arginine/glycine genotype, which composes roughly 50% of the population, was shown by a previous retrospective study to give the same positive response to albuterol as the glycine/glycine genotype.

Patients with mild asthma carrying either the glycine/glycine genotype or the arginine/arginine genotype were tested. Although those with the arginine/arginine genotype showed a better immediate response to albuterol, respiratory function and control of symptoms progressively worsened in those receiving albuterol compared with those receiving placebo. Additionally, patients with this genotype showed improved respiratory function during the run-in period when albuterol was restricted. Thus long-term treatment with albuterol appeared to be inappropriate for patients with the arginine/arginine genotype. In contrast, patients with the glycine/glycine genotype showed improvement in respiratory function and control with albuterol compared with placebo.[2]

Long-term treatment with albuterol appeared to be inappropriate for patients with the arginine/arginine genotype

These results raise interesting questions about the desirability of long-term use of beta$_2$-agonists in all asthma cases. The implication is that genotype analysis would be valuable in determining the most effective treatment for asthma sufferers: those with the arginine/arginine genotype might get more relief if prescribed a non-beta$_2$-agonist drug for regular, long-term use. Further trials examining the long-term effects of other beta$_2$-agonists, and the influence of other polymorphisms, are in progress.

As with the example of mercaptopurine, this study delivers a clear safety message. What was considered to be a widely applicable drug for a common condition may cause problems in individuals with specific genotypes. This makes a fairly strong case for genotype testing prior to prescription, and for genotype determination as a part of the clinical trial process.

What was considered to be a widely applicable drug for a common condition may cause problems in individuals with specific genotypes

Multiple genes may influence safe drug dose

Unfortunately, such clear-cut relationships between drug response and genotype are not always apparent. This should not be a complete surprise when we bear in mind

the many different proteins that may influence the level of active drug in the body, as well as the ability of the target protein itself to respond to the drug. There is still much to be learned about the multiple pathways through which a drug may be metabolised, and their interrelationships *in vivo*. The cytochrome P450 proteins are involved in the metabolic clearance of a large number of drugs, and illustrate some of these issues.

The cytochrome P450 group of enzymes is liver-based. One of the group, cytochrome P450 CYP2C9, is involved in the metabolism of a number of drugs (e.g. tolbutamide, phenytoin, warfarin and ibuprofen) and the activation of the anticancer drugs cyclophosphamide and ifosphamide (Table 2). At least 21 different alleles of CYP2C9 have been identified, varying in amino-acid identity at specific positions in the protein, although most are quite rare. The alleles appear to produce cytochromes with activity lower than the wild-type CYP2C9*1 allele, and one (CYP2C9*6) produces a cytochrome with no activity. Individuals who are homozygous for CYP2C9 are called extensive metabolisers since they can metabolise their substrates at a normal rate. Heterozygotes are called intermediate metabolisers, whereas individuals carrying two low-activity alleles are known as poor metabolisers (Fig. 1).

Laboratory measurement showed that there was a range in activities of the protein variants produced by other CYP2C9 alleles, resulting in large variations in the theoretical clearance rates of the drugs tested, with in-vitro drug clearance rates between high- and low-activity alleles ranging from 3- to 34-fold.[3] Thus, even when the same gene is involved, different drugs may exhibit various responses to the genotype. Thus, determining what alleles of CYP2C9 a patient possesses could help ensure that he/she receives a safe and effective drug dose.

Determining what alleles of CYP2C9 a patient possesses could help ensure that he/she receives a safe and effective drug dose

Examination of the effect of specific genotypes on particular drugs reveals a more complex picture, however. In an investigation in which CYP2C9 variants *1, *2 and *3 were tested for in-vitro conversion of the anticonvulsant valproic acid to hepatotoxic and inactive metabolites, there was found to be a significant loss in activity of the *2 and *3 variants compared with the *1 normal (wild-type) variant.[4] It would be easy to predict from such in-vitro studies that individuals with a genotype that includes two low-activity alleles (*2,*2; *2,*3; or *3,*3) should be given reduced-drug regimens. The situation is complicated, however, by the involvement of CYP2C9 in conversion of the drug to a hepatotoxic metabolite. Studies with liver microsomes indicate that the low-activity genotypes show no significant difference in metabolism of the drug compared with the wild-type genotype [4]. If this result is correct, it may be because there is a built-in overcapacity in the wild-type individual, and hence a reduction in the activity of this cytochrome does not significantly change the ability of the cell to metabolise valproic acid. Alternatively, there may be other metabolic clearance pathways that are able to take over the role of this cytochrome when necessary.

Complexity is also found in genotyping for warfarin response. Poor metabolisers require reduced doses compared with patients homozygous for the wild-type allele of CYP2C9. However, the genotype for another gene, VKORC1, must also be taken into account when assessing dosage. Thus, detection of the genotype for a single gene may not be sufficient to provide reliable genetic data for assessment of the correct drug regimen.

Detection of the genotype for a single gene may not be sufficient to provide reliable genetic data for assessment of the correct drug regimen

Another example of multiple-gene influence is conversion of cyclophosphamide and ifosphamide to their active forms. As already mentioned, this is carried out by CYP2C9, but a clinical study has shown the value of genotyping for other cytochrome P450 types, CYP3A4 and CYP3A5, as well as the glutathione S-transferase gene, in predicting treatment outcome.[5]

Identifying genes involved in drug response

Clearly, examination of known drug target and metabolising proteins for the effect of different alleles on efficacy and safety can produce valuable results. However, as we have also seen there may be unknown processes in the cell, presumably regulated by other genes, that modify the effect of these alleles. So, how can these other genes be identified? The answer is through use of a genetic approach in which a broad genetic profile of those individuals who respond differently to a drug is compared with that of normal responders. Elements of the profile that appear to correlate with drug sensitivity can then be used to identify the responsible genes. This is the method that has been extensively used to identify genes associated with genetic disorders, and is now employed in searches for genotypes associated with specific drug responses. Using this approach, potential blind alleys in which allelic variants of known genes appear to cause no variation in drug response can be avoided.

The genetic approach depends on the use of 'polymorphic genetic markers'. The human genome

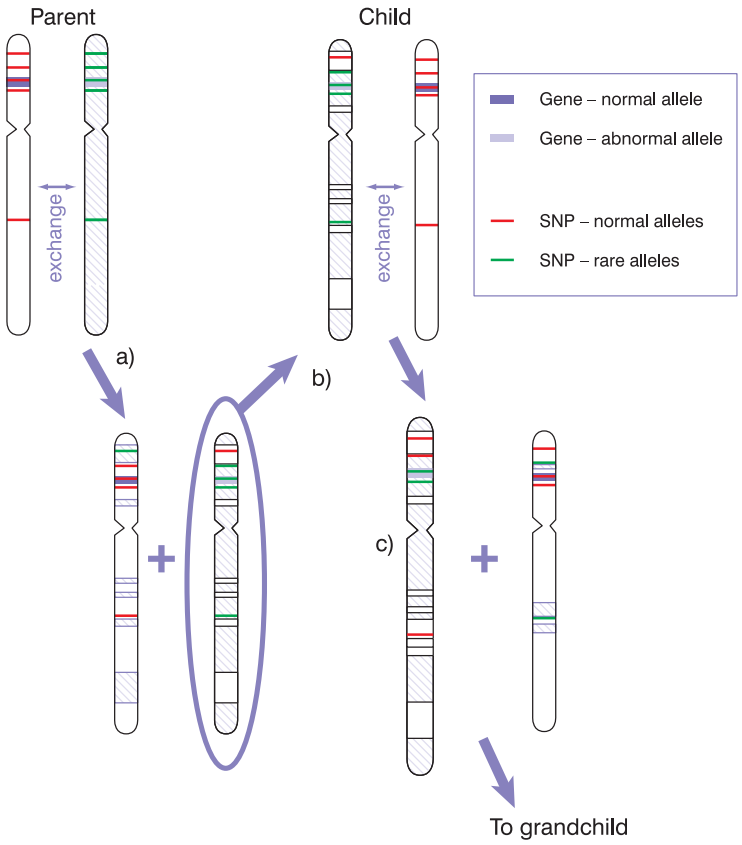

Fig. 3. Genetic linkage between markers and genes can be used to identify the gene and allele responsible for a particular characteristic. In this example single-nucleotide polymorphism (SNP) markers are distributed at points in the chromosome at varying distances from a gene: a) exchange of DNA between the parent's two copies of the chromosome produces chromosomes each with a mosaic of sequences from the two original chromosomes; b) one of the chromosomes is passed on to the child, where it joins a copy from the other parent. DNA exchange again occurs between chromosome copies. At each exchange the probability that a gene copy will lose the DNA sequences with which it originated increases. However, sequences (e.g. marker alleles) close to the gene are more likely to remain on the same chromosome. Thus the gene in the chromosome c) only retains original marker alleles which are next to, or within, the gene.

consists of DNA molecules containing more than 3 billion bases in a linear sequence, divided into 24 chromosomes (autosomes 1–22 and the sex chromosomes X and Y). Despite the size of the sequence, the probability of a specific 20-base or longer sequence occurring more than once in the genome is extremely small, and the longer the specific sequence segment is, the lower the probability of its recurrence. As described above, small changes in sequence may occur and be inherited. If such a change has occurred at a particular position in the genome, that position will be found as two (or more) different versions or alleles within the population (i.e. the position is polymorphic). Its inheritance can be tracked in the same way as inheritance of a gene, by locating the polymorphism using the sequence around it and testing by DNA analysis. This provides a means of differentiating genetically between individuals that does not rely on genes or their effects. If the position on the chromosome is known, the DNA segment is more valuable as a marker since it informs us of the state of the specific region of the chromosome on which it lies.

Why is this useful? During the formation of egg and sperm, segments of DNA are exchanged between the pairs of identical chromosomes that we have inherited from our parents. The resulting 'shuffled', or recombined, chromosomes are each allotted to a single sperm or egg, so that during fertilisation a single set of chromosomes from the sperm are added to a single set in the egg (Fig. 3). In this way, although we only receive a single set of chromosomes from each of our parents, within these sets we receive a selection of gene alleles from both sets of grandparents.

In the shuffling process, random segments of DNA are exchanged between homologous chromosomes, rather than, for example, single gene segments. This means that specific alleles of DNA sequences (gene or non-gene) that lie close to each other on the chromosome are likely to be inherited together through the generations. This is known as genetic linkage. In this way, a specific allele of a genetic marker can be inherited together with the particular allele of a gene that it is close to or within, although it may have nothing to do with the gene or its function. By looking for marker alleles that are co-inherited with genetic disorders, and then identifying the genes that are located close to, or at, the marker, it has been possible to identify the genes associated with many inherited disorders. This process has been greatly facilitated by the completion of the Human Genome Project and consequent cataloguing and locating of human genes and genetic markers. When genotypes responsible for specific drug responses are sought, the same approach is used.

A specific allele of a genetic marker can be inherited together with the particular allele of a gene that it is close to or within

The role of single-nucleotide polymorphisms in gene identification

Clearly, if this approach is to be used, a large selection of suitable markers has to be available for testing. Markers need to be present at frequent intervals throughout the genome so that every gene will be close enough to, or include, a marker, so that genetic linkage can be detected. Current efforts are centred on markers known as SNPs (single-nucleotide polymorphisms). These are locations at which alternative bases may be found at a single position in the base sequence of the genome. There are usually two known alleles (i.e. two alternative bases), and the most useful SNPs are those at which one allele occurs at a frequency of less than 5% in the population. SNPs have now been identified and recorded in databases for about every 1000 bases in the genome, both within and outside gene sequences.

For genetic linkage studies, SNPs which lie in the region of genes that are candidates for drug response can be selected from the database. The value of SNPs in investigating the genetic basis for variability in drug response has been demonstrated in many studies. Two are discussed here.

The value of SNPs in investigating the genetic basis for variability in drug response has been demonstrated in many studies

Variations in response to allopurinol

Allopurinol is used to treat gout, Lesch–Nyhan Syndrome and recurrent kidney stones. However, it can cause severe reactions, leading, in some cases, to death. There is some evidence for inheritance of hypersensitivity to the drug, and in a recent study 823 SNPs were tested for linkage with susceptibility to severe side effects.[6] In line with other work suggesting an immunogenic mechanism, the data indicated an association with genes in the major histocompatibility complex region of the genome. Genes in this region code for proteins that play a central role in regulating the immune response. Further work showed that an allele of a specific human leucocyte antigen (HLA) gene conferred a high risk for adverse drug reactions in the tested Han Chinese population. Alleles of three other HLA genes contributed to a lesser extent. This points the way towards increasing safety in drug prescribing by patient genotype testing prior to use.

The HLA-B genotype has also been shown by SNP analysis to be important in determining response to abacavir, which is used in the treatment of HIV. In this case, drug hypersensitivity is linked to possession of the HLA-B*5701 allele.[7]

Variations in response to statins

Statins are used to lower the risk of cardiovascular problems through reduction of low-density lipoprotein (LDL) cholesterol levels. Response to these drugs varies considerably between patients. The genetic basis of this response was investigated in a study in which SNPs linked to 10 genes with a possible influence on LDL metabolism were tested for association with low drug efficacy in patients.[8] The data indicated that two SNPs in the gene for the enzyme targeted by statins (3-hydroxy-3-methylglutaryl coenzyme A reductase) were strongly linked to a low efficacy of statins in reducing LDL cholesterol. Thus, the SNP-based investigation led to the identification of alleles critical to the effect of statins that had not previously been detected. Although this example does not involve drug toxicity, drug efficacy is, of course, relevant to safety issues.

Population differences in allele frequency

Although SNPs are a valuable tool in the identification of genes and proteins that influence drug safety, and can be used in screening programmes, there are some constraints that have to be considered. Because of the nature of inheritance, populations that have been relatively isolated from each other until recently may have different frequencies and collections of alleles. This can be seen among the CYP2C9 alleles. Whereas the frequency of the CYP2C9*2 allele is 8–19% in Caucasians, it is 1–4% in African-Americans and it does not appear to be present in Asians. The frequency of the CYP2C9*3 allele is 6–10% in Caucasians, 0.5–1.5% in African-Americans and 1.7–5% in Asians. In all cases, the major allele found in the population is the wild-type CYP2C9*1.[9]

There is good and bad news associated with this clear illustration of population genetics. The good news is that if alleles causing poor drug responses are restricted to a particular population (or are more frequent in that population), screening for these alleles may be considered necessary only for that population. The bad news is that all populations in which the drug is likely to be used will have to be separately studied for the frequency of the allele in question, so that population-associated risks can be assessed. This has clear cost implications. Despite these qualifications, SNPs are now used routinely in many investigations, and are being introduced into patient screening programmes.

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Making use of pharmacogenetic data

Assuming that the principle of a strong drug response–genotype link can be proven, where do we go from there? Before data supporting an association between drug response and specific genotype can be implemented in the healthcare environment a number of hurdles have to be overcome:

1. The evidence should achieve a specific standard.
2. The relationship should make a significant addition to our ability to predict drug response (in some cases response can be adequately predicted without genotyping).
3. The knowledge of an association between a particular genotype and drug response is useless in practice unless a test to distinguish genotypes is available for use in diagnostic laboratories.
4. Information on the association and its implications in prescribing need to be presented to the prescriber in a standardised and easily understood manner. It has been observed that whereas prescribers rarely read directions, lawyers always do.

Guidelines are now available from the US Food and Drug Administration for the standard of evidence for linkage between genotype and drug response when this is intended for use in decisions regarding therapy.[10] Although SNP data alone can show clear linkage, the requirement is for reproducible data that include an understanding of the biochemical relationship between the protein(s) coded by the gene(s) identified and the drug. For those drugs metabolised by CYP2C9, for example, these relationships are well characterised since cytochrome P450 has long been known to be associated with drug metabolism and many biochemical studies have been carried out. Additionally, a validated genotyping test must be available. Bearing this in mind, it is almost certain that the development of drugs will now be accompanied by an exploration of their pharmacogenetic effects.

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Once there is sufficient evidence for an association between genotype and drug response, the current route taken for inclusion of this data in labelling depends on risk. In any case, if there is an issue regarding the effectiveness in, or risk to, a subgroup of the population (i.e. the group possessing the alleles in question), the labelling of the drug should indicate not only this but also the tests necessary to assess the patient. The nature of the information and recommendations will depend on the specific drug and its effects, and on local regulations.

For example, the anticancer drug irinotecan can be considered at different levels. This drug is normally inactivated by the enzyme UGT1A1. The allele UGT1A1*28 has low enzyme activity, and therefore causes higher levels of drug exposure than the normal allele. Therefore, labelling would include a recommendation for a lower starting dose in patients known to be homozygous for the UGT1A1*28 allele. Furthermore, a warning that such individuals are at increased risk of neutropenia after starting treatment would be required. Patients with one normal and one UGT1A1 allele (heterozygotes) have intermediate enzyme activity and therefore the warning should indicate a possible but reduced risk for these individuals. Information regarding the test required for genotyping would also be needed.[11]

Conclusions

Since drugs interfere with the processes that occur in the cell, it is not surprising that their use can lead to adverse effects when applied at inappropriate levels. This has been recognised for many years. Factors such as body weight or surface area, other medical conditions and drugs prescribed, and age are commonly considered when determining drug dosage. What is becoming increasingly obvious is that, with some drugs, genotype can also influence the safe dose for an individual. Over the past decade there has been rapid accumulation of data on the genetic content of the human cell, and the development of fast and economic techniques for DNA analysis. Because of this we are now at a stage where genotype–drug interactions can be investigated relatively easily and the information used to reduce risk in drug therapy.

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However, through the very nature of drug development and testing, the proportion of individuals in the population who are sensitive to a drug by genotype is small. Because of this, statistically significant data on drug sensitivity by genotype can be difficult to acquire, making validation of the relationship difficult. One means of increasing the database would be to gather information systematically from healthcare professionals regarding adverse reactions to drugs, preferably with some genotype data.

Although the knowledge of genetic influence on drug response is an important development for drug safety, there are some natural limits to its application. As suggested, there is little sense in applying genotype testing to drugs for which there is already an adequate means of predicting dose outcome. Nor is it useful to determine genotype where there is no significant difference in drug response *in vivo*, even when alleles causing abnormal drug metabolism *in vitro* are present. However, there are cases where genotyping has obvious benefits for safety. Drugs used in cancer chemotherapy are a good example. The window of safe and effective dosage is very narrow for these drugs, and genotype can be a critical factor in setting the maximum safe level.

Perhaps equally important is the influence that the knowledge of possible genetic effects will have on the development and testing of new drugs. Use of genetic data and techniques has the potential for aiding the development of drugs against new and better-defined targets than those produced in the past. This may well lead to the development of safer drugs simply through better knowledge of mechanisms and identification of possible side effects and toxicity. However, what is also true is that drug development will now have to include consideration of effects on the range of genotypes or genes that are implicated in the drug action and clearance. The knowledge accruing from this should make safe drug therapy a much more likely prospect.

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