The response to drugs and environmental chemicals varies with genotype. Some patients react well to drugs, while others may not benefit, or may even respond adversely. Individuals also experience different reactions to environmental agents, such as allergens. The sequencing of the human genome and the large-scale identification of genome polymorphisms have provided opportunities for understanding the genetic basis for individual differences in response to potential toxicants: an area of study that has come to be known as toxicogenetics. In this article, we discuss the potential applications and implications of this evolving branch of toxicology.

Variability in the Human Genome

Only approximately 0.1% of the three billion base pairs of DNA that comprise the human genome varies in sequence between individuals. However, the information encoded in this small portion of the genome can have profound effects on biological makeup. The most dramatic examples are seen with inherited disorders, where small alterations in gene sequence can result in premature death, or severe disability (Alberts et al., 2002; Habener and Williams, 2002; King et al., 2002). Moreover, there is increasing evidence that susceptibility to clinical conditions not previously considered to be genetic diseases such as obesity, cancer, and cardiovascular disease—may have significant genetic components. Genetic makeup also affects recognition of and responses to xenobiotics, and, as a consequence, relative susceptibility to induced adverse health effects. An appreciation of how genetic variation is able to determine, or at least influence, relative resistance to toxicants has the potential, eventually, to transform risk assessment from a population-based paradigm (with attendant uncertainties and the need for safety factors to accommodate possible, but unknown, interindividual differences in susceptibility) to a determination of relative risk tailored to individual subjects (Roses, 2000).

Much of the variability in the human genome is in the form of single nucleotide polymorphisms (SNPs). The publication of the human SNP map in 2001 by a public consortium represented the largest-ever analysis of human genome variability (Sachidanandam et al., 2001). The consortium found that SNPs occur, on average, about once every 1000–2000 bp. More than 99% of these are biologically silent, while the effects and biological significance of the remainder vary according to their position within the genome. Table 1 contains the Internet addresses of Web sites containing more information on genome polymorphisms and SNP mapping.

Polymorphisms can affect biological function in several ways (Fig. 1). SNPs that fall within the coding region of a gene can give rise to a protein that has an amino acid substitution, or is truncated, causing a change in activity, localization, or stability. For example, SNPs of this kind occur in the CYP2D6 gene (Marez et al., 1997). Similarly, polymorphisms that induce shifts in translational reading frames will lead to the synthesis of proteins with altered amino acid sequence and, perhaps, activity (for an example, see Iida et al., 2001). Nucleotide alterations in the regulatory regions of a gene can also have a significant impact on the integrity of protein function. Polymorphisms in promoter regions may change the regulation and level of expression of a protein, whereas those that fall near intron-exon junctions may cause alterations in mRNA splicing (Kuehl et al., 2001). More dramatic polymorphisms involving larger segments of the genome include gene deletions, gene conversions, and gene duplications.

Although it is important to catalog the biochemical consequences of polymorphisms, perhaps the most exciting application of SNP maps is identification of those alleles that are associated with heritable traits. A disease-associated SNP that falls within a gene can provide information on the mechanistic basis for disease, while an SNP that is in linkage disequilibrium, with a genetic allele that confers disease predisposition may be used to identify susceptible individuals. The aim of SNP mapping is to identify polymorphisms that are associated
verifiably with heritable traits (Roses, 2000), and naturally this can include those heritable traits that influence relative susceptibility or resistance to toxicants.

**Genome Polymorphisms and Drug Discovery**

Advance identification of those patients that will benefit from a drug, those that will experience no advantage and those that may develop undesirable side effects, is clearly desirable. The study of relationships between genotype and drug activity has been termed pharmacogenetics (Nebert, 1999; Park and Pirmohamed, 2001; Roses, 2002; Wolf et al., 2000). The principle is elucidation of the molecular genetic bases for interindividual variations in susceptibility to the anticipated beneficial (or adverse) effects of a drug. The corollary is that the same strategy and technology can be used to explore differences in sensitivity or resistance to other xenobiotics to which exposure occurs. The response of subjects during the course of clinical trials of new drugs is frequently diverse. Ideally, a drug will be of therapeutic benefit to all patients, without the risk of unintended adverse effects. However, subsets of patients often do not benefit from the drug, and others may even experience unpredicted effects (Pirmohamed and Park, 1999).

Differences in response to drug treatment are commonly due to polymorphisms in genes encoding proteins that influence the pharmacokinetic parameters of a foreign compound. For example, polymorphisms that cause alterations in xenobiotic-metabolizing enzymes can influence the response to a number of drugs, including diazepam, omeprazole, and carbemazepine (Ingelman-Sundberg et al., 1999; Park and Pirmohamed, 2001; see below). Failure to inactivate a drug, due to a defect in a specific metabolizing enzyme, can result in delayed clearance and precipitate unwanted side effects. Conversely, failure to activate a

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**TABLE 1**

<table>
<thead>
<tr>
<th>Resource</th>
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<tr>
<td>The SNP Consortium (TSC)</td>
<td><a href="http://snp.cshl.org/">http://snp.cshl.org/</a></td>
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<tr>
<td>National Cancer Institute</td>
<td><a href="http://press2.nci.nih.gov/sciencebehind/snps_cancer/snps_cancer0.htm">http://press2.nci.nih.gov/sciencebehind/snps_cancer/snps_cancer0.htm</a></td>
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</tr>
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<td>GenesSNPs at University of Utah</td>
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<td><a href="http://egp.gs.washington.edu/">http://egp.gs.washington.edu/</a></td>
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<tr>
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<td><a href="http://gai.nci.nih.gov/">http://gai.nci.nih.gov/</a></td>
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<td>Japanese SNP database</td>
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<tr>
<td>The Sanger Institute Human Genome Project site</td>
<td><a href="http://dbsearch.sanger.ac.uk/HGP/help/overview.shtml">http://dbsearch.sanger.ac.uk/HGP/help/overview.shtml</a></td>
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<tr>
<td>Cytochrome P450 allele nomenclature</td>
<td><a href="http://www.imm.ki.se/CYPalleles/">http://www.imm.ki.se/CYPalleles/</a></td>
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**FIG. 1.** The effects of genome polymorphisms on protein function. Polymorphisms that fall within the coding region of a gene may alter protein activity by causing amino acid substitution, production of an altered splice variant, or protein truncation (A). Polymorphisms involving larger segments of DNA include gene deletion (B) and gene duplication (C) and result, respectively, in loss of protein activity and increased protein activity.
pro-drug results in loss of efficacy. Candidate drugs can be screened using polymorphic, as well as normal forms, of metabolizing enzymes, thereby avoiding the development of inappropriate candidates. Such information is potentially valuable also in the management of clinical trials, allowing selection of subjects based on an appreciation of likely efficacy and susceptibility to possible adverse drug reactions.

**Genome Polymorphisms and Toxic Responses**

The initial metabolism of drugs and other foreign chemicals is carried out, predominantly, by the cytochrome P450 (CYP) family of enzymes (Danielson, 2002; Nebert and Russell, 2002). The observation that many of the 63 human CYP genes present are polymorphic (Ingleman-Sundberg *et al*., 1999; Nelson, 1999) provides a rationale for interindividual differences in responses to xenobiotics. Polymorphisms in the CYP2D6 gene have been studied extensively. The enzyme encoded by this gene is required for the metabolism of up to 25% of all drugs and, presumably, a similar proportion of environmental chemicals (Ingleman-Sundberg *et al*., 1999). Several CYP2D6 polymorphisms cause alterations in enzyme levels and/or activity. A proportion (6%) of Caucasians possesses two null alleles of the CYP2D6 gene, and as a consequence is unable to metabolize and clear certain drugs, resulting in unwanted side effects. This “poor-metabolizer” phenotype can be caused by the production of an inactive or unstable protein resulting from gene deletion, gene conversion, or single-base-pair mutations. Other polymorphisms result in the production of variant CYP2D6 proteins with decreased activity, leading to more subtle alterations in the rate of drug clearance. Certain other polymorphisms result in increased levels of the CYP2D6 enzyme due to gene amplification, with some individuals having as many as 13 copies of the gene (Johansson *et al*., 1993). Patients with unusually high levels of CYP2D6 enzyme (“ultra-high metabolizer” phenotype) can experience idiosyncratic responses to foreign chemicals, and may clear certain drugs so rapidly that therapeutic target levels are not achieved with normal doses.

Polymorphisms in another drug-metabolizing enzyme, CYP2C9, cause differences in the response to the anticoagu- lant, warfarin. Extensive variation in the way in which patients respond to a given amount of warfarin makes the setting of safe and effective dose levels problematic. However, recent studies have shed light on the genetics of warfarin metabolism (Aithal *et al*., 1999; Taube *et al*., 2000). The CYP2C9 enzyme metabolizes the most active enantiomer of this compound: S-warfarin. Polymorphisms in the CYP2C9 gene encode amino acid substitutions that result in enzymes with a reduced ability to metabolize S-warfarin and, consequently, individuals with this genotype maintain unusually high levels of S-warfarin in serum. The reduced metabolism and clearance of the active enantiomer in these patients means that they require less war- farin to achieve therapeutic serum levels. Importantly, levels of the drug that are safe to patients carrying normal CYP2C9 alleles may cause adverse effects in those with polymorphic alleles (Park and Pirmohamed, 2001). Therefore, genotyping of patients to determine which CYP2C9 alleles they carry may be used to set the optimum dose of drug for each individual patient. Understanding the genetic basis for variability in the response to other drugs will no doubt allow dose setting to be tailored in a similar fashion.

Polymorphisms in other gene families can also affect individual responses to foreign compounds. Most chemicals are metabolized via a pathway that involves “phase I” (functionalization) and “phase II” (conjugation) (Rushmore and Kong, 2002). The CYP enzymes participate in phase-I metabolism. Variations in activity of phase-II enzymes, such as for instance glutathione S-transferases (Strange *et al*., 2001), can also affect response to drugs and environmental chemicals. Polymorphisms in other protein families are also likely to affect the response to xenobiotics, and included among these are drug transporters, plasma membrane and nuclear receptors and ion channels. Although few polymorphisms in these gene families have been reported to date, it is likely that biologically important genetic variations do exist.

**Identifying Genes that Confer Susceptibility to Adverse Effects**

Traditional methods for the identification of genes that confer susceptibility to disease or adverse drug reactions employ pedigree analysis and positional cloning (Birren *et al*., 1998). The aim of these techniques is to first identify conserved chromosomal regions in the genomes of susceptible individuals, and to then use finer mapping to home in on candidate genes. As a result of the publication of a high-density SNP map, opportunities exist to develop more rapid, more economical, and more effective methods. It is now possible to identify susceptibility genes by mapping SNPs that are present in the genomes of individuals with the trait of interest. The identification of a gene responsible for common, late-onset Alzheimer’s disease demonstrated the power of this approach (Lai *et al*., 1998), and can be used here as a paradigm for the principles involved.

The e4 allele of the gene encoding the apolipoprotein E4 (APOE4) has been shown to increase the risk of developing Alzheimer’s disease (Roses, 1996). Lai and colleagues (Lai *et al*., 1998) sought to determine whether SNP mapping could be used to identify candidate susceptibility genes. SNPs were mapped over a large chromosomal region encompassing the APOE4 gene to determine whether this gene could be identified independently as a risk factor for Alzheimer’s disease. The frequency with which each SNP occurred in the region of interest was measured in patients with Alzheimer’s disease and in an age-matched, disease-free control population. Statistical analyses were then employed to identify individual SNPs that
were overrepresented in the genomes of patients compared with controls. In effect, these analyses revealed regions of DNA within the chromosomal segment of interest that were similar in subjects with Alzheimer’s disease. The genomes of patients tended to have a group of linked SNPs clustered around a region containing only two genes: APOE4 and APOC1. This confirmed the previous identification of the APOE4 e4 allele as a possible risk factor for Alzheimer’s disease and demonstrated the power of SNP-mapping approaches for the identification of susceptibility genes (Lai et al., 1998). A similar approach has been used recently to localize gene(s) conferring susceptibility to Crohn’s disease to a 250 kbp region spanning the cytokine gene cluster on chromosome 5q31 (Riou et al., 2001).

The SNP-mapping approach has also been used successfully to identify the genetic bases for idiosyncratic adverse drug reactions. Abacivir is a reverse transcriptase inhibitor used for the treatment of human immunodeficiency virus (HIV) infection. Adverse reactions to this drug occur in approximately 4% of patients (Hetherington et al., 2002). Symptoms include fever, rash, and respiratory problems. A hypothesis-driven approach was used to design a genetic analysis based upon an appreciation that adverse reactions to Abacivir have an immune pathogenesis. As a result, attention focused on 100 selected polymorphisms from candidate genes known to play influential roles in the development and/or regulation of immune responses. The results revealed that polymorphisms in the gene encoding tumor necrosis factor α (TNF-α) and in genes within the major histocompatibility complex (MHC) were associated with adverse reactions to the drug (Hetherington et al., 2002; Mallal et al., 2002). Encouragingly, the success of this study suggests that hypothesis-driven, SNP-mapping approaches can be used successfully to identify the genetic bases for adverse reactions to xenobiotics. However, the abacivir mapping study may not have been successful were it not for the availability of a large group of sensitive subjects; 4% of patients exhibited drug-induced adverse health effects. Often, idiosyncratic adverse reactions to drugs are observed only sporadically in a very small number of individuals. In these cases, genetic analysis of sensitive patients may lack sufficient statistical power.

Examples of the use of SNP mapping cited above relied on some degree of prior knowledge of the genomic region(s) conferring susceptibility. The human SNP map has also made global, nonhypothesis-driven mapping of susceptibility genes possible. In theory, SNP mapping of susceptible and nonsusceptible individuals could be conducted over the entire genome, giving an unbiased view of genetic risk factors. The obstacles to this approach presently are technical and economic. Based on current knowledge, approximately 200,000 SNPs would have to be analyzed for complete genome coverage (Roses, 2002). However, efforts are in train to identify a smaller set of nonredundant and clinically informative SNPs that can be analyzed efficiently and economically using a single assay.

Defining the Genetic Basis for Differential Susceptibility to Exogenous Agents

Molecular genetic approaches have begun to elucidate the hereditary basis for differences in susceptibility to certain inhaled environmental toxicants. SNPs in the promoter region of the tumor necrosis factor α (TNF-α) gene and the interleukin 1 (IL-1) gene cluster that alter cytokine mRNA expression levels have been associated with chronic inflammatory diseases. A recent study revealed a relationship between these SNPs and susceptibility to silicosis in coal miners exposed to crystalline silica (Yucesoy et al., 2001). Three hundred twenty-five ex-miners with varying degrees of lung silicosis, and 164 miners without the disease, were studied. The presence of the TNF-α (-238) variant was associated with severe silicosis, whereas the IL-1 receptor antagonist (+308) and TNF-α (-380) variants were elevated in subjects with both mild and severe forms of the disease.

Other examples are provided by polymorphisms conferring susceptibility to allergic sensitization and/or allergic disease. Allergy may be defined operationally as the adverse health effects that may result from the stimulation of specific immune responses; of particular relevance to immunotoxicology being responses to chemicals and proteins encountered in the workplace or in the general environment. Although there is little evidence for heritable susceptibility to chemical-induced allergies, such as allergic contact dermatitis, it is well established that inheritance of an atopic phenotype predisposes toward the development of IgE-mediated allergic responses to proteins. The picture is complicated, because atopy is polygenic in nature, and susceptibility is also influenced markedly by environmental factors. In addition, evidence is emerging that certain discrete polymorphisms may influence the pathogenesis of allergy. Two recent examples serve to illustrate the point. One study implicated polymorphisms in N-acetyltransferase 2 (NAT2). It was found that slow acetylation status, dictated by NAT2 allelic inheritance, was associated with a significantly higher incidence of IgE antibody-mediated food allergy (Gaworiska-Szklarz et al., 2001). The other example is provided by interleukin 10 (IL-10), a cytokine with diverse effects on the immune responses. Recent investigations by Karjalainen et al. (2003) suggest that although the IL-10 polymorphisms appeared not to determine susceptibility to adult allergic asthma *per se*, there was an association between certain haplotypes and serum IgE levels and numbers of circulating eosinophils in asthmatic subjects.

Toxicogenetics and Toxicogenomics Provide Complementary Information

The opportunities and applications offered by toxicogenetics are frequently discussed in tandem with those promised by
toxicogenomics. In the context of our discussion, it is relevant to draw a clear distinction between these disciplines. Genetics is the study of relationships between heritable differences in genome sequence and biological phenotype, while the term genomics has come to describe the study of alterations in gene expression associated with biological responses. Toxicogenetics, therefore, describes consideration of stable and heritable alterations in the genome that are able to influence the relative susceptibility of an individual (or group of individuals) to the adverse health effects that may result from exposure to an exogenous material. Toxicogenomics, on the other hand, describes analysis of gene-expression changes induced in a biological system by exposure to a xenobiotic (Afshari et al., 1999; Farr and Dunn, 1999; Gant, 2002; Nuwaysir et al., 1999; Orphanides et al., 2001; Pennie et al., 2000; Ulrich and Friend, 2002). The two disciplines are linked; polymorphisms that alter biological function may change the spectrum of genes regulated in response to a toxicant. In this way, toxicogenetic differences can underpin variations in toxicogenomic response. In the future, toxicogenomic data from global gene-expression profiling and toxicogenetic data from genome-wide SNP mapping, when considered in concert, will facilitate the identification of genes and pathways that determine relative susceptibility to potentially toxic materials.

Ethical Considerations

One of the ultimate goals of toxicogenetics is the identification of genetic markers (e.g., SNPs) that will be predictive of relative sensitivity to drugs and environmental chemicals associated with adverse effects. Although, in practice, the widespread application of this may be some way off, it is important that possible ethical issues relating to the use of genetic information are identified, acknowledged, and debated. The collection and storage of personal genetic information is controversial and has attracted much public attention. Should individuals be tested for predisposition to highly penetrant genetic disorders? If so, who should have access to this information? What are the implications if insurers and potential employers have access to an individual’s genetic information? Moreover, if one could define alleles that conferred increased susceptibility to xenobiotics that may be experienced in particular occupations, could this, or should this, inform recruitment strategies? These are issues to be debated among the scientific fraternity and the general community. It has been suggested that potential problems with the ethical use of this kind of genetic data can be minimized by selecting SNPs that are of pharmacogenetic and toxicogenetic value, while avoiding those that predict genetic disease (Roses, 2002). In this respect, toxicogenetics can learn from the forensic sciences: the widely used technique of “genetic fingerprinting” uses a small number of highly polymorphic, unlinked genetic markers that have no known implications to the health of an individual.

Concluding Comments

It is clear that advances in our appreciation of the genetic bases for idiosyncratic responses to chemicals will have practical applications in drug discovery, hazard identification, and risk assessment and will more fundamentally contribute to our understanding of the health consequences of the interaction of xenobiotics with biological systems. Potential benefits include (a) accelerated discovery of gene polymorphisms associated with idiosyncratic toxicity, (b) the identification of genetic markers for the prediction of adverse reactions to drugs, (c) the definition of genetic markers for drug efficacy, and (d) characterization, at the molecular level, of interindividual differences in susceptibility to xenobiotic-induced adverse health effects that can be used as the basis for development of more differentiated and more accurate assessments of human risk.

Economic obstacles to genotype-based medicines, driven by a fear of market segmentation, must be addressed and overcome in order for these potential benefits to be realized and exploited. Similarly, ethical issues relating to the collection and storage of personal genetic information must be considered carefully to ensure that data are used appropriately.

REFERENCES


