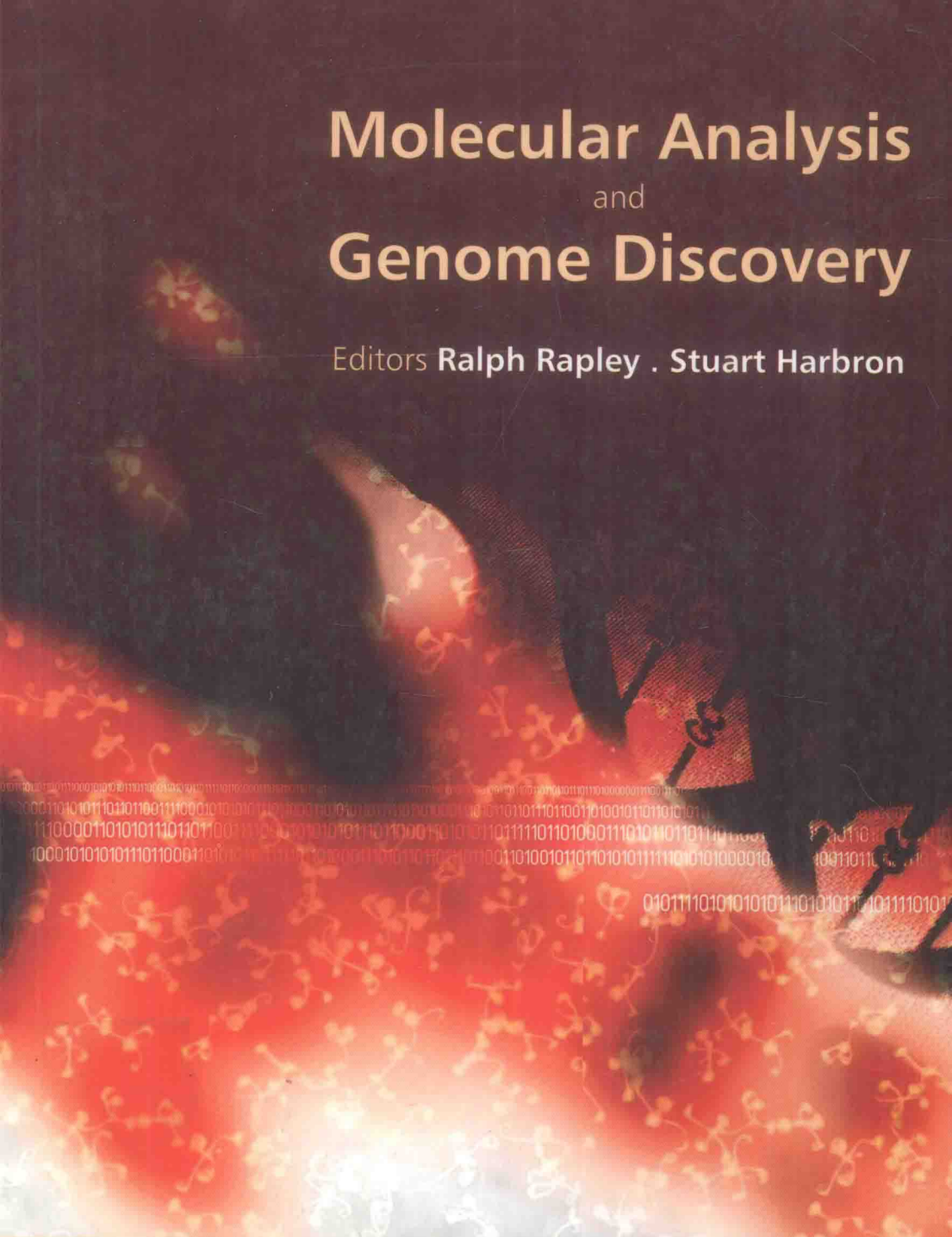
Molecular Analysis and Genome Discovery

Editors **Ralph Rapley . Stuart Harbron**



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Molecular Analysis and Genome Discovery

Preface

There can be no doubt that the face of diagnostics and drug discovery has changed beyond recognition over the last decade. Advances in the techniques of molecular analysis, and the ever-increasing use of automation, especially high throughput approaches, have paved the way for new, rapid and more reliable diagnostic tests. Completion of the Human Genome project has, in addition, been a startling achievement that can only accelerate the discovery of new genes and biomarkers for disease processes.

The genomics revolution promises to replace current prescription medicines with a new class of much more potent and efficacious medicines. The success of this revolution, the process of converting the claims of personalized medicine into an armamentarium specific for each patient, is dependent on continuing advances in molecular analysis and genome discovery.

This book aims to bring together these two aspects and show how the rapid advances in molecular analysis are bringing new tools and approaches to genome discovery. In combination, these provide the impetus for the development of new diagnostic techniques.

Following a historical overview of pharmacogenetics and pharmacogenomics by Werner Kalow, the first part of the book has detailed explanations of the latest techniques from experts in the field, copiously illustrated with examples from their own laboratories.

Chapters from Jörg Dötsch and Elaine Lyon present alternative approaches to quantitative and real-time PCR techniques and how they may be used in tumour and mutation detection.

Ivo Gut covers Genotyping and SNP analysis, and issues such as improving the economy of this approach are explored. Emerging techniques and the promise of high throughput SNP genotyping methods are presented.

Paal Andersen and Lars Larsen provide a comprehensive overview of mutation detection and screening. Methods for mutation scanning are also discussed.

Pyrosequencing is an exciting new approach to genomic analysis, and its principles and applications are explained and illustrated by Elahe Elahi and Mostafa Ronaghi.

Development of DNA-based microarrays have been a key area over the last decade, both from a research point of view and commercially. Their fabrication

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and use are expertly covered in chapters from Magdalena Gabig-Ciminska and Andrzej Ciminski, and from Janette Burgess; a chapter from Jon Terrett's group focuses on the complementary chip-based proteomic technology.

Ciara O'Sullivan *et al.* discuss the powerful approach enabled by aptamer technology. Uniquely she relates how aptamers may be selected for use in both proteomic *and* therapeutic applications.

Kin-Ying To describes how a number of these molecular techniques may be applied to an analysis of differential gene expression.

The remaining chapters focus on the latest approaches to the identification of new target compounds, and Roberto Solari provides an excellent overview of the multi-stage drug discovery process.

Huang and Jong provide an interesting insight into potentially powerful approaches to analysing global features of microorganisms as they relate to the elucidation of new antibacterial agents.

The discovery of new target compounds based on an analysis of known structural motifs and elements is covered by Kan *et al.* and David Winkler, while Hudes, Menon and Golemis contribute a chapter investigating the impact of drugs on protein–protein interactions.

In compiling *Molecular Analysis and Genome Discovery* we have sought to combine both current and emerging approaches to analysing DNA, proteins and genomes, whilst not losing sight of the challenging outcomes required for successful drug discovery. In achieving this aim we have benefited from calling on the expertise of a distinguished and creative group of contributing authors; we have thoroughly enjoyed working with them.

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Contents

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1

Pharmacogenetics and Pharmacogenomics: An Overview

W. Kalow

Pharmacogenomics has recently arisen from the well established science of pharmacogenetics; in fact, both names are sometimes mixed or interchanged by students of abnormal drug effects. The current overview will therefore start with a discussion of pharmacogenetics, its origin and its coverage; this will be followed by a description of the new aspects which pharmacogenomics brings into the study of interaction between genes and the drugs given to patients.

The origins of pharmacogenetics

Pharmacogenetics emerged from the combination of the old sciences of genetics and pharmacology. Sir Archibald Garrod (1931) anticipated the occurrence of individual differences in human reaction to drugs and environmental chemicals in his book *Inborn Factors in Diseases*. J.B.S. Haldane (1949) studied biochemical individuality and also predicted the occurrence of unusual reactions to drugs. By that time, a few genetic differences in drug response had been seen.

Snyder (1932) had described the inheritance of a deficient ability of some people to taste phenylthiocarbamide (PTC), fundamentally a pharmacologic observation. Sawin and Glick (1943) noticed a genetic lack of atropine esterase in some rabbits, a deficiency that restricted their consumption of belladonna-containing plants. During World War II, the generally safe antimalarial drug primaquine

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caused haemolysis in many American soldiers, but only in soldiers of African descent; later, the event was shown to be due to deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), a deficiency frequent in Africans, where the mutant tended to cumulate because it protected its carriers from malaria (Beutler 1993). The new exciting tuberculosis-fighting drug isoniazid was seen (Hughes *et al.* 1954) to have neurological effects in some people, those later shown to have a familial deficiency of its destroying enzyme, N-acetyltransferase. Also in the 1950s, the new muscle-paralysing drug succinylcholine, used during anaesthesia, killed some patients while most had no trouble; the cause was found to be a mutation which inactivated plasma cholinesterase, the succinylcholine-destroying enzyme (Kalow 1956).

Several physicians and scientists felt stimulated by these reports. A committee of the American Medical Association invited the geneticist Motulsky (1957) to write a paper entitled 'Drug Reactions, Enzymes, and Biochemical Reactions' for their journal. The geneticist Vogel (1959) in Germany coined the word 'Pharmacogenetics' in a paper describing 'Modern Problems of Human Genetics'. I was in the process of summarizing all pertinent findings in a book (Kalow 1962). Pharmacogenetics was now an established entity.

The initial progress of pharmacogenetics

As time went by, more pharmacogenetic discoveries were made. Denborough (Denborough and Lovell 1960; Denborough *et al.* 1962) reported the occurrence of excessively high body temperatures (hyperpyrexia), rigidity and death among various family members in response to general anaesthetics; this condition is now called malignant hyperthermia. Dundee *et al.* (1962) emphasized the paralytic effect of barbiturates in cases of hepatic porphyria. Aebi *et al.* (1961) observed in Switzerland cases with a genetic lack of catalase activity, cases as seen before only in Japan. Von Wartburg *et al.* (1965) reported genetic variation of alcohol dehydrogenase. Further pharmacogenetic cases were reported in the following years, but, in addition, some of the older reports were extended. For instance, Kalow *et al.* (1970) showed that malignant hyperthermia represented a biochemical defect in skeletal muscle that caused the muscle to respond abnormally to caffeine, thereby making *in vitro* tests and predictions of this condition possible.

All these cases represented relatively rare observations and therefore did not dramatically affect the practice of medicine. This changed with the report on familial failures of the oxidation of debrisoquine, a sympatholytic, antihypertensive drug. Dr R.L. Smith (1986), a medical investigator in England, took the drug personally for experimental purposes, but suffered a marked and extensive hypotensive episode. He therefore started to investigate the metabolism of the drug, found the metabolic defect, and reported the findings in an excellent publication (Mahgoub *et al.* 1977). A short time later, Eichelbaum *et al.* (1979) in Germany

reported that some people could not metabolize sparteine, an anti-arrhythmic drug. Subsequently, other investigators (Bertilsson *et al.* 1980; Inaba *et al.* 1980) found that the same liver enzyme metabolized both debrisoquine and sparteine. All measurements were based on the drug/metabolite ratio in urine.

The medical interest in this topic was stimulated by several facts. Oxidation is an important and widespread metabolism in human and animal bodies. The defect was not rare but obviously affected quite a few people; that is, it was a polymorphism, a frequently occurring genetic alteration. The enzyme was a cytochrome P450 (CYP2D6), an important class of drug-metabolizing enzymes. It was soon found that the enzyme metabolized many drugs, presently thought to be as many as 20 per cent of all clinically used drugs. Medline cites currently almost 2000 publications dealing with CYP2D6.

The usual clinical effect of the deficiency is an over-response, or toxic response, to any drug that is not metabolized by CYP2D6 (Meyer 2001). However, if a drug needs metabolic activation, an under-response, or lack of response, can also occur. For instance, the debrisoquine-metabolizing enzyme is also the one which converts codeine into morphine. If the enzyme lacks, this conversion does not take place, and codeine is not a pain-killing analgesic (Dayer *et al.* 1988).

Molecular genetic methods enlarged pharmacogenetics

As we can see, pharmacogenetics was in the beginning entirely concerned with functional differences in drug response or drug metabolism, that is, *phenotype* differences which could be directly observed or measured in family members. Pharmacogenetics became much enriched when new methods, described in the following chapters, allowed a direct look at the DNA of mutant genes, thereby determining *genotype*.

The use of the methods of molecular genetics disclosed many kinds of mutations (Stockley and Ray 2001), revealed as changes of nucleic acids. The most frequent variants are SNPs, *S*ingle *N*ucleotide *P*olymorphisms, indicating simple exchanges of two nucleotides; many genes carry several, often many SNPs (Grant and Phillips 2001 and see Gut; Chapter 4). For instance, about 130 SNPs have been described for G6PD, the enzyme glucose-6-phosphate dehydrogenase (http://www.bioinf.org.uk/g6pd/). However, some SNPs do not alter the amino acid of the derived protein, and therefore do not affect protein structure and function; they are called *silent*. Some mutations may change reaction rates, substrate binding, sometimes affecting only selected substrates. There are also other complexities. For instance, absence of an enzyme activity could be caused by any one of four kinds of mutations: frame shift, splicing defect, strip codon or gene deletion. In addition, genetic tests sometimes revealed gene duplication or even multiplication, thereby greatly decreasing the action of dependent drugs. Table 1.1 lists the number of alleles among members of the drug-metabolizing