Indian J Physiol Pharmacol 2007; 51 (1) : 7–28

REVIEW ARTICLE

HUMAN GENETIC VARIATION AND PERSONALIZED MEDICINE

SURAKSHA AGRAWAL* AND FAISAL KHAN

Department of Medical Genetics,
Sanjay Gandhi Post Graduate Institute of Medical Sciences,
Raebareli Road, Lucknow – 226 014

(Received on July 17, 2006)

Abstract : Human genome sequencing results revealed an insight into the role of human genetic variation behind differential susceptibility of human diseases, differential response to pharmacological agents and presence of varied phenotypes. This leads to the concept of personalized medicine. In the present review we have discussed the objectives and approaches for carrying out pharmacogenomics and pharmacogenetics studies. The review also incorporates the major findings categorizing the common diseases on the basis of genetic profiles and ethnic information and in establishing personalized disease diagnosis, drug responses and treatment modalities based on the genetic determinants. Overall an attempt has been made to highlight the importance of studying the genetic profiles of an individual in biomedical and pharmacogenomics research.

Key words : polymorphism pharmacogenomics pharmacogenetics genetic ancestry candidate gene

INTRODUCTION

As soon as the two independent sequence drafts of the human genome were surfaced (1–2), it was clearly established that the two ‘genomes’ that each of us carry, inherited from our parents, most often differ from each other, and from the genomes of other humans (3). These genetic differences interacts with the environmental factors in producing varied phenotypes, inducing differential human susceptibility to diseases and leading to differential response to pharmacological agents (4–5). Thereafter, the scientific literature got flooded with the reports of role of genetic variation in disease susceptibility, the age of onset, its severity and finally, its treatment (6–9).

The findings were more important in the light of the fact that the response of patients to drug therapy varies widely from one individual to another worldwide, both in terms of the beneficial effects of the drug as in the occurrence of serious and often unpredictable side-effects or adverse drug reactions. The clinical consequences of adverse drug reactions range from patient discomfort to serious illness, which requires hospitalization and may even result into

*Corresponding Author : Phone : 091-522-668004-8, Ext. 2338, 2346, 2347, 2339; Fax No.: 091-522-6680973/6680017; Email : suraksha@sgpgi.ac.in

death. Adverse drug reactions accounted for 2.2 million hospitalizations and over 100, 000 deaths in the USA in 1994, making adverse drug reactions the 4th-6th leading cause of death in the USA. In the UK, 1 in 5 hospitalizations can be attributed to adverse drug reactions (8). None of the factors including age, gender, body weight, patient health, disease status, diet, smoking, alcohol, exercise and drug interactions provided any guarantee that a given treatment will be effective or well tolerated in a given patient. However, the projects of human genome sequencing and human genome diversity and the subsequent reports compelled the gentists and pharmacologists all over the world to believe that the major cause for variability in drug responses lies in a patient's genetic make-up (8–13).

The findings lead to the emergence of the filed of 'Personalized Medicine'; it simply means the prescription of specific treatments and therapeutics best suited for an individual and avoids the trial and error approach of conventional medicines. Pharmacogenomics, Pharmakokinetics and Pharmacoproteomics are the basic foundation of personalized medicine while molecular diagnostics being the major tool.

1.1 Pharmacogenetics and pharmacogenomics

The study of genetic variants or polymorphisms that influence drug responses has given rise to a principal challenge in the analysis of these data mainly, the difficulty in linking information about the variation in human genes to the variation in drug response (pharmacogenetics) and to understand how interacting genes determine individual drug responses (pharmacogenomics) (7, 13). The joint disciplines of pharmacogenetics and pharmacogenomics are interdisciplinary and collaborative fields requiring the cooperative efforts of research and clinical scientists (Fig. 1).

Pharmacogenetics is defined as the study of variability in drug responses due to heredity. Pharmacogenomics examines the role of the entire genome in both disease susceptibility and drug response, in an attempt to identify specific genes that are associated with specific diseases and that may be the targets for new drugs (10). Pharmacogenetic variations can be classified into three main types according to their mechanism of action. The first is pharmacokinetic, in which genetic variants are associated with drug transporters and metabolizing enzymes, and lead to alterations in the uptake, distribution and elimination of drugs. The second is pharmacodynamic, in which genetic variation occurs in the drug target or a component of the target pathway leading to altered drug efficacy. Pharmacodynamic targets include receptors, ion channels, enzymes, transducer and regulatory proteins, and immune molecules. A third mechanism is idiosyncratic, in which genetic variants result in unintended actions of a drug outside its therapeutic indication (11–13).

The role of genetic variation in drug response or adverse reaction defines the science of Phamacogenetics (13), and it actually started in 1950s with the emergence of human biochemical genetics. The best cited example is occurrence of hemolytic anemia due to G6PD deficiency. Way back in 1957, Motulsky published a report about

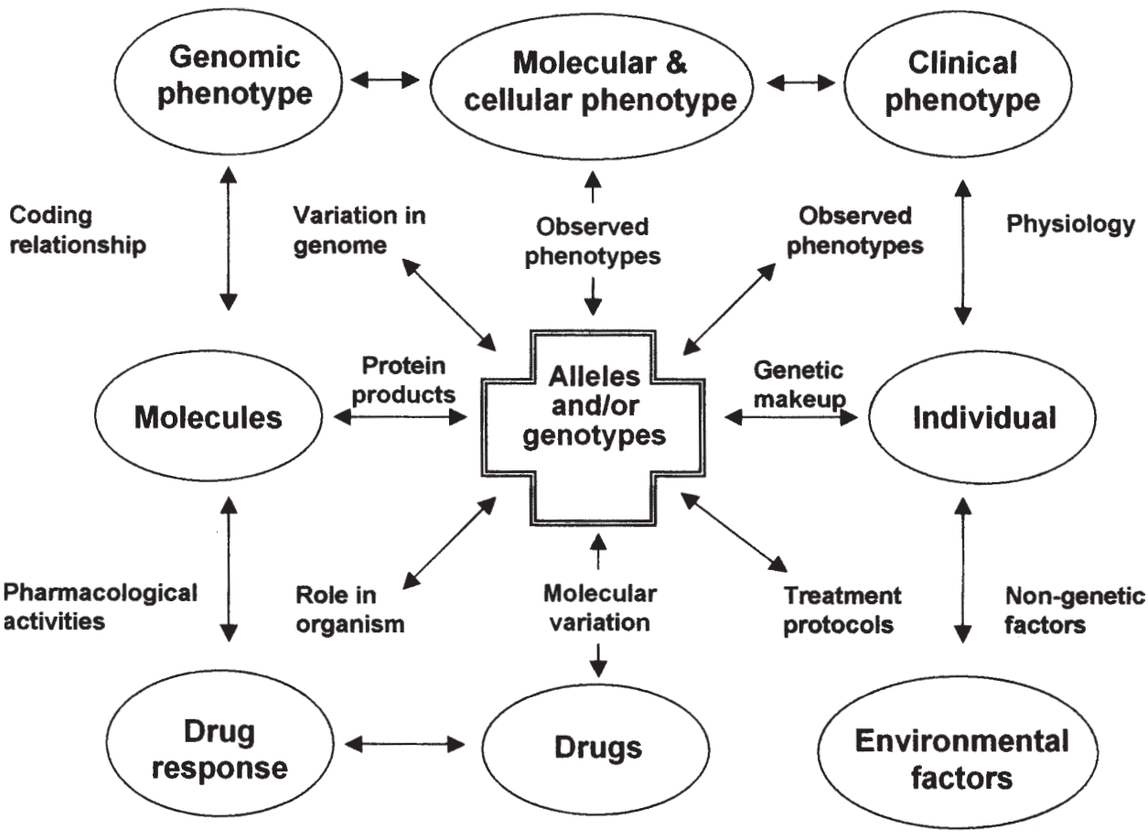

Fig. 1: Interdisciplinary nature of pharmacogenetics and Pharmacogenomics.

drug reactions, enzymes and biochemical genetics. The term 'pharmacogenetics' was basically coined by Friedrich Vogel of Heidelberg, Germany in 1959 (14). In late 1960s, Vesell showed remarkable similarity of disposal for several drugs in monozygotic twins who share 100% of their genes as contrasted to dizygotic twins who share only 50% (15). The term 'pharmacogenomics' was re-introduced in 1990s with emergence of the Human Genome Project and the development of the human genome sciences.

Latest high throughput technology has added newer dimension to the search of multiple genes and their expression affecting drug responses. Recent developments in technology have revealed large number of new mutations even for diseases like hemolytic anemia. A 24 bp deletion of nucleotide 953–976 in the exon 9 of the G6PD gene causes the G6PD deficiency. The parents were found to be heterozygous for this mutation and appropriately advised on the condition and the importance of taking folic acid regularly (16). Search for characteristic cellular DNA abnormalities in disease is now beginning to guide

construction of therapeutic drugs acting on disease specific DNA mutations (17). A somatic mutation in chronic myelocytic leukemia responds to the drug Gleevec in almost 100% of cases (18).

Similarly, Long-QT syndrome is a clinically and genetically heterogeneous syndrome characterized by lengthening of the QT interval and increased dispersion of the ventricular repolarization on surface electrocardiogram and a propensity to malignant ventricular arrhythmias, torsade de pointes and ventricular fibrillation, which may lead to sudden cardiac death. Long-QT syndrome mostly affects adolescents and young adults with structurally and functionally normal hearts and is caused by aberrations in potassium and sodium ion channels (19). Standard therapies for long-QT syndrome include correction of the underlying cause, alleviation of the precipitating factors, magnesium sulfate, isoproterenol, antiadrenergic therapy (beta-adrenergic receptor blockers, left cervicothoracic sympathectomy), cardiac pacing, and implantable cardioverter defibrillator. The potential therapies include sodium channel blockers (mexiletine, flecainide, lidocaine, pentisomide, phenytoin), potassium, potassium channel activators (nicorandil, pinacidil, cromakalim), alpha-adrenergic receptor blockers, calcium channel blockers, atropine, and protein kinase inhibitors. There is individual variation and different therapies are being tried in response to the personal genetic variation (19).

1.2 How genetic variation affects susceptibility to a disease or drug action

Humans are considerably more similar to each other than other species as any two randomly chosen humans differ at ~1 in 1000 nucleotide pairs, whereas two random chimpanzees differ in ~1 in 500 nucleotide pairs (20). Nevertheless, there are ~3 billion nucleotides on haploid human genome, therefore on an average two humans differ at ~3 million nucleotides which is ~0.1–0.2% of the haploid human genome (21). Most of these variants are neutrals but still common variants are present in coding and regulatory regions of genes that alter the amino acid sequences and gene expression respectively (22).

Majority of the polymorphisms observed are single nucleotide polymorphisms (SNPs), short Tandem repeat polymorphisms (STRPs) or insertion-deletion polymorphisms (indels). If these polymorphisms are present in the coding or the regulatory region of various genes whose products participates in the pathology, physiology or treatment of a particular disease, then their presence might lead to individual variation both for the occurrence of the disease or the action of drug against it.

An ideal example of such genetic control is evidenced by the identification of polymorphisms in the gene regulatory region of cytokines, chemokines and growth factors that correlates with intra individual variations in actual cytokine or chemokine production (23–25). As these polymorphisms segregate independently, each individual is a mosaic of high, moderate and low cytokine producing phenotype. Presence of such high, moderate and low producing SNPs in the regulatory domain of cytokines leads to their differential, responses against immunosupressors in case of organ and bone

marrow transplantations and against anti-inflammatory drugs in various diseases like asthma.

A vast majority of such polymorphisms are found in the regulatory promoter regions, while various others are found in the intronic, exonic and untranslated regions. The promoter gene polymorphisms may disrupt or abolish transcription regulatory elements such as those involved for NfKB and STATS (signal transducers and activators of transcription) (26–27). All these regulatory elements regulate RNA polymerase binding, influencing the rate at which gene is transcribed into mRNA. Intronic variation may affect enhancer/silencer sequences and certain polymorphisms may alter architectural transcription factor binding elements (27).

Furthermore, the differential distribution of the genetic variation in different populations owing to various factors like mutations, selection, and genetic drift and to some extent migrations and mating patterns also leads to the differential susceptibility to diseases and differential responses to drugs (5–6). Natural selection is the result of population variation among individual genotypes in their probabilities of survival and/or reproduction. Earliest evidences include selection of Heterozygotes of hemoglobin A/S polymorphism for having greater resistance to malaria. Similarly, FOXP2 gene has shown a two amino acid difference in human and chimpanzees suggesting the role of this gene in evolution of speech and language in modern humans (28). Strong molecular evidences are available for the selection of activity of G6PD locus to confer resistance to malaria (29) and TNFSF5 in response to infectious agents (30). Other potent examples of genes having a signature of selection are Dufry antigen (31), drug metabolism- CYP1A2 (32) and alcohol metabolism- ADH1B and ALDH2 (33).

Random genetic drift occurs due to a finite number of individuals participating in the formation of the next generation. This process is responsible behind the genetic differences between African and non-African populations (3). The impact of drift on differential susceptibility of diseases could be seen from the fact that sickle-cell anemia is found in wide range of people including Hispanics and inhabitants of north-western India (34), but the blacks of South Africa do not carry sickle cell traits (34–35). Similarly, high frequency of null allele of CYP2D6 makes Arabs more capable of transforming codeine into the active form morphine.

1.3 Approaches to pharmacogenomics and pharmacogenetics studies

An ideal pharmacogenomics or pharmacogenetics study involves various crucial steps.

(a) Identification of appropriate candidate genes whose expression may influence drug action or disease pathogenesis.

(b) Identifying all observed polymorphisms with complete sequence.

(c) Carry out case-control studies in various populations to identify the association of a genetic variant with disease or drug response, and

(d) Assess the effect of the genetic variant on the expression profile of the gene.

These four steps together with the elements of pharmacokinetics and pharmacodynamics, i.e. the variability in pharmacokinetics of the concerned drug and variability in association with drug and/or genetic variant on the phenotype which completes the overall requirement for an ideal study of personalized medicines. Such study require a database that can model key elements of the data, acquire data efficiently, provide query tools for analysis and deliver the resulting system to the scientific community (7). Pharmacokinetics includes absorption, distribution, metabolism and elimination of a drug. Pharmacodynamics includes pharmacological effects and clinical response leading to toxicity and efficacy for a particular drug or metabolite (7).

(A) Candidate gene selection

The candidate gene approach for discovering genetic markers use experimentally derived a priori knowledge about a disease or a drug. Scientists do background research, employing both public and proprietary databases, to identify appropriate candidate genes whose expression may impact drug action or disease pathogenesis. Candidate genes are commonly selected based on metabolic pathways, molecular targets, biological response pathways and/or disease risk. Generally, the genes are ranked based upon their perceived likelihood of being involved in the drug response. The stronger candidates can then be tested first. If these top candidates fail to explain sufficiently the variation in drug response, additional candidate genes can then be tested.

A classic example of this approach is the variation in individual responses to the anti-leukemia drug, 6-mercaptopurine (38–39). Thiopurine drugs are metabolized, in part, by S-methylation catalyzed by thiopurine S-methyltransferase (TPMT). Most people metabolize the drug quickly. Some individuals, with a genetic variation for the enzyme TPMT, do not. Consequently, they need lower doses of 6- mercaptopurine for effective treatment as normal doses can be lethal. Patients with very low or undetectable TPMT activity are at high risk of severe, potentially fatal hematopoietic toxicity when they are treated with standard doses of thiopurines (38). As human TPMT activity is controlled by a common genetic polymorphism, it is an excellent candidate for the clinical application of pharmacogenetics. The point mutations in the TPMT gene that cause the loss of TPMT activity can be detected by a fluorescently labeled amplified DNA which is hybridized with oligonucleotide DNA probes immobilized in gel pads on a biochip. The specially designed TPMT biochip can recognize six point mutations in the TPMT gene and eight corresponding alleles associated with TPMT deficiency : TPMT(*)2; TPMT(*)3A, TPMT(*)3B, TPMT(*)3C, TPMT(*)3D, TPMT(*)7, and TPMT(*)8. In such instance identification of the candidate gene TPMT was the most decisive step and information about its action on thiopurine drugs helped in its recognition as a potent gene responsible for varied pharmacological action (39).

(B) Identification of genetic variants

The pharmacogenomics studies require a detailed model of genomic sequence, in order to represent accurately DNA sequence

data, gene structure and polymorphisms in sequence, much more than simply storing the DNA sequence. Recent advents of automated DNA sequencing (40), denaturing HPLC (41), mass spectrophotometry (42), array based re-sequencing (43), automated fragment size analysis and SnapShot PCR have not only increased our repertoire of mutation but have also provided a high throughput techniques for quick and reliable genotyping. However, still the choice of technique varies from RFLP, ARMS, gene-scanning and re-sequencing to dHPLC depending upon the type of marker being studied.

Single nucleotide polymorphisms, SNPs, are the most abundant and the simplest form of DNA variation. A SNP originates with a mistake in copying a single nucleotide letter in a DNA sequence. The mistake is simply that one letter gets replaced with another. There are different effects that this change may have. Their effects vary from being silent to lethal (44). There are 1.42 million known SNPs found at a density of one SNP per 1.91 kb. This means that more than 90% of any stretch of sequence 20 kb long will contain one or more SNPs. The density is even higher in regions containing genes. The International SNP Map Working Group2 estimates that they have identified 60,000 SNPs within genes (‘coding’ SNPs), or one coding SNP per 1.08 kb of gene sequence. Moreover, 93% of genes contain a SNP, and 98% are within 5 kb of a SNP (44).

The main use of the human SNP scoring is to dissect the contributions of individual genes to various diseases that have a complex and polygenic basis. Variation in genome sequences underlies differences in our susceptibility to, or protection from, all kinds of diseases; in the age of onset and severity of illness; and in the way our bodies respond to treatment. These gene variants lead to tissue and organ incompatibility, affecting the success of transplants (22).

Large number of genes and their variants are implicated in the success of renal transplantation; as a result many impending and potent methods have also become available for genotyping of SNPs and STR loci in last few decades. These methods vary from sequence specific primer (SSP) based typing to automated DNA sequencing of the whole fragment where the SNP is located. Other flourishing methods include restriction fragment length polymorphism and amplification refractory mutational system (ARMS). However, Studies covering underlying genetic component of a diverse number of conditions require systems for genotyping large numbers of SNPs. In this regard, a mini-sequencing method known as Snapshot and Taqman probes based real time PCR are ideal techniques, where former is effective for small-scale investigation while later is used for high throughput analysis (22).

(C) Case-control based association studies

Association studies offer a potentially powerful approach to identify genetic variants that influence susceptibility to common disease as well as response of a particular drug (45–47). However, such studies are often plagued by non-reproducible results (48). In principle, the inconsistency may be due to false positive studies, false negative studies or true variability in association among different populations

(47, 49). It has been widely accepted that undetected population stratification in case-control studies are major reason behind the false positive associations (50). Association studies can yield large numbers of spurious associations if population subgroups are unequally represented among cases and controls (51). The problem of population stratification increases when cases or controls are derived from a metropolitan city where people of numerous ethnic backgrounds live together or there has been a genetic mixing of two or more groups.

Knowler et al (52) have studied the Gm polymorphism of human immunoglobin IgG gene and type II Diabetes among 4290 Native American individuals belonging to Pima and Papago Indians. They found a highly significant negative association. However, once they included the criteria of genetic ancestry then they found that frequency of Gm polymorphism is much higher and occurrence of type II diabetes is very low in people of no American Indian ancestry then foil American Indian ancestry.

With recent advancement in human genetic variation studies (53–54), there has been a broad consensus on the fact that genetic knowledge of population sub-structuring and stratification is an essential requirement for proper selection of controls and for identifying disease pre-disposing alleles that may differ across ethnic groups (55–56). An interesting example in this regard would be of Indian populations. Various reports cites the possible Caucasian genetic ancestry of north Indian populations reflected by the high frequency of western Eurasian and central Asian NRY haplogroup-Rla Rib, R2 etc (57–58). However, both FVL mutation (A2086G) and prothrombin gene mutation (C10965T) that have been reported to be associated with CAD and thrombotic events among Europeans (59) were not found in any of the samples in our study on normal as well as CAD and RSA patients from north India (60). Similarly, the ACE DD genotype has been associated with hypertension and CAD (61) but no such results are found among north Indians (62). However, same ACE DD genotype has been found highly associated (P=0.0001; OR 25.7) with patients of end stage renal disease (63). Such studies ultimately help in deciding the doses and type of anti-hypertensive therapies.

Similarly, studies based on successful association of MTHFR (677C/T) SNP with increase in the homocysteine levels due to production of thermpliable MTHFR enzyme (64–65) leads to deciding a quantity and dosages of folic acid supplementation. Another interesting examples is strong association Factor V-leiden SNP (1691G/A), and Prothrombin gene mutation (20210G/A) with hyper coagluable state (66–67) that helps in to fix a proper anti-coagulatory therapy. Furthermore, immunosuppressor drugs like cyclosporin and tacrolimus carry a narrow therapeutic range and a wide inter individual variation in its pharmaco-kinetics (68). The most important factor that affects their inter-patient variability is p-glycoprotein (p-GP), a product of multi drug resistant gene-1 (MDR-1) that uses these immuno-suppressors as its substrate (69). Four important SNPs have been studied in MDR-1 gene namely 129T/C in exon lb, 1236C/T in exon 12, 2677G/T in exon 21 and 3435C/T in exon 26, where later three SNPs are more frequently found in general

population (69–71). Exon 21 SNP was found highly associated with tacrolimus dose as TT homozygous showed 40% higher dose requirement of tecrolimus but not for cyclosporin doses (70). Exon 26 SNP-3435C/T is reported to be associated with increased expression of MDR1 gene in CD56+ NK cells (70–7l). However, the report regarding its role in drug kinetics is contradictory. More focused and targeted studies on such genes will lead to individualization of drug treatment.

(D) Expression analysis

Expression analysis both at mRNA and protein level is an ardent need to understand the contribution of genetic variant in stimulating or suppressing the expression and thereby assessing the exact role on the drug kinetics. This provides the precise mechanism involved in particular individual behind the success or failure of a drug. In fact, this particular step is the most essential step before declaring a particular SNP to be involved in the variability of drug responses (22). Various recent studies have done such analysis where the information generated by the genotypic profile of the patients was substantiated by the mRNA expression and ELISA based assays (23). Such an exercise can clear the controversy about the role of the SNPs in the peleotropic molecules like cytokine IL6 that acts as both pro and anti-inflammatory.

(E) Modeling of phenotypic data and its correlation with the genotype

Finally, it is mandatory to have phenotype data revealing molecular, cellular and clinical profile to correlate with the presence of a genetic variants and its effect on the expression of the related gene. Molecular and cellular phenotype data include enzyme kinetic measurements, such as binding, catalysis and inhibition constants for particular drugs, cellular drug processing rates, homology modeling of three-dimensional structures and pharmacodynamic assays (7). Clinical phenotype is perhaps the most difficult data to model and link with genomic and molecular/cellular phenotypic data. Clinical phenotype data include basic pharmacokinetic measurements (such as drug absorption, distribution, elimination and metabolism) as well as pharmacodynamic profiles, which currently include pulmonary, cardiac and psychological function tests, and cancer chemotherapeutic side effects.

Apart from the most successful candidate gene approaches, whole genome analysis and use of various statistical parameters is also often used for pharmacognetic studies.

Whole genome analysis

A whole genome analysis is effectively the opposite of a candidate gene based study. Rather than focusing on a set of genes that are already expected to be involved in how patients respond to treatment with a drug, one attempts to test the entire genome. The obvious benefit of this approach is that genetic loci that no one ever expected to be involved in the response may be revealed, potentially adding greatly to the understanding of the drug, the disease or general biology. Another reason to take this approach is if a good candidate gene list cannot be assembled based on prior knowledge.

There are reasons why most Pharmacogenomic studies are not done this way. First, the expense is likely to be prohibitive, although some researchers have pooled their samples, which raises several issues. Second, as a compromise to reduce the expense, each gene generally has to be measured with lower resolution. For example, by choosing only one SNP to represent a gene, the power to detect the true genetic factors involved in drug response is reduced. Third, given the vast number of genetic loci that have to be tested to cover the entire genome (several thousand to several hundred thousand), the true findings are likely to be swamped by a sea of false positives. It is generally a bad experimental design to have many more tests than subjects and most clinical trials are limited to a few thousand subjects. (72). This final problem will remain regardless of how significantly genotyping costs may decrease as new technologies are developed. One of disease tried widely is hypertension. Increasingly, detailed characterization of human molecular genetic variation will facilitate the use of genetic information in preventing, diagnosing, and treating common diseases (73). One promising application is the identification of genetic variants influencing responses to drugs used to lower blood pressure (BP) and prevent target-organ complications of hypertension. This update on gene markers to guide antihypertensive therapy highlights polymorphisms recently reported to predict inter individual differences in response to antihypertensive medications. However, single-site variation in most genes makes only a small contribution to differences in BP response, and, after all known genetic and environmental predictors have been considered, most variation in responses still remains unexplained. Advancing beyond our current “trial-and-error” approach in selecting drug therapy in individual patients will undoubtedly require whole-genome approaches to discover additional, novel genetic pathways influencing drug response. In addition, larger samples will be required to more fully characterize genetic variation within candidate genes and to consider the joint effects of gene-gene and gene-environment interactions. Eventually, knowledge of genetic variants that influence BP responses may allow more individualized tailoring of therapy to optimally reduce BP and target-organ damage (73).

Statistical approach

After all the hard clinical and laboratory work, the data produced must be interpreted to determine which genetic variants affect which clinical variables and under what conditions (74–76). This part of the process is called statistical analysis. Regardless of the experimental design (candidate gene or whole genome, prospective or retrospective), statistical analysis of the resulting data is a tremendous challenge. While it is important to have a statistical analysis plan in place before launching into a Pharmacogenomic project (74), the nuances of how to interpret the results may not be apparent until the data are produced. Here we present a few of the common challenges with which one must deal when working with genetic data, which extend beyond the complexities inherent in any clinical study.

1. Genetic variables are not simple categorical or quantitative traits like gender or age. The variation found in a single gene is instead quite complex. Often, there may be a thousand of

different ways to break the variation into smaller pieces, or “markers” that range in scale from a single SNP variant to a whole-gene haplotype. Because the true causative aspect of a gene variation could occur at any level, we would like to test all of these possibilities. Many of these alternative markers within a gene, however, are correlated with each other to some degree but differ nevertheless. Determining the most plausible association may be more involved than just finding the best “P-value”.

2. For each genetic variable, there may be several ways, or “genetic models”, for how the variable relates to a clinical trait. Is the genetic marker dominant or recessive? and does this increases or reduces the efficacy or side effects of the drug? These genetic models are all possible. Examining the variation found in the clinical trait may suggest which models are likely. For instance, if there are two distinct responses to the drug, dominant and recessive models would be reasonable models to consider. However, in most cases, the pattern of the variation in treatment response does not allow a clear determination of which models to consider, so multiple models should be tested.

3. As many genes are found on the same chromosome, sometimes very close together, a finding for one gene may really reflect the effect of a neighboring gene. All positive findings must be checked for this possibility. If a neighboring gene is plausibly related to the clinical trait (e.g., the response to a drug), secondary analyses, perhaps requiring additional laboratory work, may be required to identify the gene that is really involved in the response.

4. Because biology is rarely as simple as one might hope, variation in multiple genes is likely to be involved in determining each aspect of drug response. Once the most promising individual genes are identified then how these components interact or combine to affect the clinical trait should be determined.

5. In a “multiple comparison testing”, a hundred of genes with a thousand markers, each tested in two models for five clinical traits, requires 1,000,000 tests. A large fraction of these tests will be found to be nominally significant in the statistical sense. Correcting for this over-abundance of positive findings require a clear understanding and determination of the complex correlations among markers, among genes, among models and among clinical traits.

6. Just as many biological characteristics, such as disease risks, differ between ethnic groups, so do the frequencies of many genetic variants. This fact raises the concern that when a genetic marker is found that predicts drug response, the marker might really just be tracking ethnicity, as would countless other genetic variants that have nothing to do with the clinical trait in which we are interested. While it is possible to control this problem by including factors, such as ethnic self-identity of patients in the analyses, the level of control provided might not be adequate and sometimes is not available or the information is

unreliable or ambiguous. One analytical tool that is now used to test for this confounding effect is known as “genomic control”. Genomic control is simply a fancy way of saying that scientists can measure a set of genetic loci that are independent of one another and unlikely to be related to the clinical traits and then use the unrelated loci to assess whether there are unsuspected ethno geographic differences between the patients with different clinical traits, e.g. those who respond well to the drug versus those who do not.

1.4 Recent diagnostic tools for studies on personalized medicine

Ramification amplification method: It is an isothermal process and sensitive and specific for amplifying nucleic acids with flexibility to analyze proteins and other small molecules on the same analytical system. This is a high throughput technique where in one go many SNPs can be amplified (77–78).

Invader assays: This technique is quite robust where perfect match enzyme substrate reactions using propriety cleavage enzymes, recognizes and cut only the specific structure formed during the invader process. It can be used directly to recognize the SNPs (79–80).

Molecular beacons: These are folded probes which gives no fluorescent signals in the folded position due to quenching of the label but upon hybridization of the molecular beacons to the target sequence the probe unfolds and the fluorescent labels emits light. Molecular beacons are able to discriminate alleles in real time PCR assays of genomic DNA. Molecular beacons are ideal tools for genetic screening and diagnosis (81).

Matrix-assisted laser desorption mean spectrophotometry: This is also known as MALDI - TOF - MS. This determines the mass of the variant DNA sequences. As large number of sequences can be detected in a single reaction so it is a cost effective technique for genotyping (82).

Biochip microarray: This is a technique which can be adapted for genomic DNA, RNA expression analysis and now even the protein chips have been developed. It has powerful data processing software, automated flow-through system and label free detection of the hybridization signals (83–84).

Nanochip technologies: This is an accurate method that allows multiplex assays and on chip amplification of DNA material directly on the Nanochip cartridge, which eliminates a time-consuming preparatory step and involves only a single step (85).

1.5 Pharmacogenomics: success story till date

As it has been clearly figured out that the evolutionary processes of mutation, migration, random genetic drift and selection resulted into differential distribution of normal genetic variation and also that of genetic variation affecting diseases and drug responses (4). The genetic variations studies based on the role of differential genomic profiles on disease susceptibility and drug response have contributed tremendously in two major areas (i) molecular sub-classification of the diseases based on the genetic profile (ii) genetic contribution to differential drug response.

Molecular sub-classification of the diseases based on the genetic profile

The impact of evolutionary forces on the differential distribution of disease genes is currently better understood in the context of the worldwide distribution of the monogenic traits (86). The distinctive examples include the parallel presence of high frequency of hemoglobin HbS allele, variant of G6PD and sickle cell anemia among sub-Saharan Africans (87–88) and Mediterranean populations or that of C28Y-HFE allele and hematochromatosis in Europeans. Each of these polymorphisms underlies a monogenic trait and hence inference of genetic ancestry can be unnecessary. However, a closer look suggest that cystic fibrosis is also found among groups of Arab and African ancestry (86, 89), similarly, sickle-cell anemia is found in wide range of people including Hispanics and inhabitants of north-western India (35). Furthermore, the blacks of South Africa do not carry sickle cell traits (35) but its occurrence in central Greece has two-fold increase than that of African Americans (35, 90). Therefore, labeling the disease only on the basis of ethnic affiliation or phenotypic occurrence can be wrong interpretation and could possess serious health consequences. The concept of genetic ancestry is a much better indicator than race or ethnicity to determine that whether one carries the marker of a genetic disease. The neutral genetic variation studies have reveled that the proportion of European ancestry in African- Americans averages ~21% (44) while that among South Indian populations is 16% compared to 84% East Asian ancestry (91).

The geographical distribution of genes associated with common diseases is more complicated as they result from complex interactions between genes and environment. There are two schools of thoughts; one view put forward the l common disease- common variant' (CD/CV) hypothesis, which states that the common genetic diseases are affected by common disease susceptibility alleles (or variants) at a few loci that exist at high frequency across ethnically diverse populations (44, 92). These alleles probably arose before population differentiation and are common across populations. Supporters of CD/CV hypothesis cite that geographic or ethnic clustering variants are mainly a phenomenon of the monogenic disorders. Furthermore, additional support to CD/CV hypothesis is provided by examples of 23 5T variant of angiotensin (AGT) gene that codes a key component of rennin-angiotensin, blood pressure regulatory pathway. This variant is present across all human groups, as high as 90% among Africans and as low as 30% among Europeans (93). The allele is associated with 20–30% increase risk of developing hypertension (94). Similarly, frequency of the null allele of CYP2D6 gene varies from 6% in Asians to 7% in Africans and upto 30% in Europeans (95). CYP2D6 encodes a member of cytochrome P450 family involved in metabolism of important drugs (13), and its null allele renders the gene product inactive to an extent that homozygous null allele individuals experience little or no analgesic effect. Therefore it has been quoted that although substantial genetic variation is there in etiology of common diseases but it is present in all populations.

The other perspective view is that of *multiple rare variant* (MRV) hypotheses

according to which the diseases are associated with substantial proportions of genetic polymorphisms and will probably be specific to groups that experience similar evolutionary forces of selection or drift (96–97). Recently, Bamshad et al, 2004 (98) has shown that a sequencing based analysis of 63, 724 SNPs in the coding and regulatory regions of 3931 human genes reveal that large number of private alleles are present in different population groups. This clearly indicates that CD/CV hypothesis was supported because only commonly occurring SNPs have been studied and even if CD/CV hypothesis is correct then also differential effects of risk allele in people with different genetic ancestry have been reported as homozygous APOE4 Asian individuals have ~5 fold higher risk of developing Alzheimer's disease even when this allele of APOE is frequent in Alzheimer patients of Africa, Asia and Europe. Several polymorphisms in the 5' cis-regulatory region of CCR5 influence the progression of AIDS and even death in HIV patients (99–100). However, one CCR5 haplotype (HHE) is associated with delayed progression of AIDS in European-Americans but with faster progression African-Americans (101). Similarly, three important variants of CARD 15 or NOD2-R702W, G908R and 1007fs have been associated with an inflammatory bowel disorder- Crohn's disease in European- Americans (102–103) but not in Europeans or Asians (104). Therefore even if the same risk allele for a complex trait is present in different group, it might be associated with different outcomes. Overall, if more is learned about the genetic bases of the complex diseases and if it is supplemented with the information about the genetic ancestry of the populations then such diseases can be divided into distinct subclasses with similar phenotypes but different underlying genetic bases.

Genetic contribution to differential drug response

The genetic differences among ethnic groups often lead to differences in drug responses. Tate and Goldstein, 2004 (6) have reviewed various genetic variants associated with drug responses and have stated that out of 42 associated genetic variants, more than 30 variants significantly differ between people of Europeans and African ancestry (Table I). An important example is that of receptor polymorphisms i.e. the β_2-adrenoceptor which has been extensively studied as a prototype of G-protein coupled receptors, and is the target for bronchodilators drugs used in the treatment of asthma. The β_2-receptor gene is known to have 9 single nucleotide polymorphisms (or SNPs) in the coding region; five are degenerate and are unlikely be functional, but four result in the amino acid substitutions within the protein at positions 16, 27, 34 and 164 (105). The functional relevance of these polymorphisms has been investigated by mimicking the polymorphisms by site-directed mutagenesis, expressing the variant receptor in host cells that lack β-receptor expression, and assessing the pharmacologic properties of these cells. Using this approach several polymorphisms have been shown alter β_2-receptor function by decreasing receptor-G protein coupling (Thr to He, position 164), increasing receptor desensitization (Arg to Gly, position 16) or decreasing receptor desensitization (Gin to Glu, position 27) (105–106). Although changes in receptor function occur *in vitro*, there has not been a

TABLE I: Differential drug responses reported in different ethnic groups.

Category of drugs	*Implication*	*Reasons*	*References*
Drugs with an indication of genetic causation			
I-Beta-adrenoceptor blockers			
Propranolol	More effective in EA than in AA in treatment of hypertension	Hypertensive and healthy controls carrying two copies	Soffowora et al (105)
Nadolol	More effective in EA than in AA for systemic hypertension	of the R389G variant in beta-1-adrenergic receptor have more response to.	Johnson et al (106)
Oxprenolol	Mean blood pressure reduction less for African-Americans than for European ancestry	Beta-adrenoceptor blockers The R389Gvariant is more frequent in Europeans than in African-Americans	Cubettu et al (114)
Bucindulol	Survival benefit only in non-African Americans		Friedman et al (115)
Drugs whose association has an essential physiological basis			
I-ACE Inhibitors			
Enalapril	Less response in AA than in EUR with congestive heart failure	Probably related to lower bio-activity of endogenous nitric oxide	Kalinowski et al (116)
Lisinopril	Response in EUR but not in AA ancestry		Exner et al (117)
Trandolapril	AA with hypertension required 2-4 times higher dose to obtain similar lowering of blood pressure to EUR ancestry		Weir et al (118) Weir et al (119)
II-Combination of two vasodilators			
BilDil	Greater efficacy in African Americans than in European ancestry with congestive heart failure		Carson et al (120)
Ill-Alpha adrenoreceptor blockers			
Prazosin	More effective in EUR ancestry than in AA ancestry for hypertension	Non-adrenergic mechanism contribute to blood pressure maintenance in AA	Cushman et al (121)
IV-Thiazide (Diuretics)			
Hydrochlorothiazide	Greater cystolic and diastolic blood pressure responses in AA than in EUR	Probably related to lower bio-activity of endogenous nitric oxides	Kalinowski et al (116) Chapman et al (122)
V-Calcium Channel blocker			
Dilpiazem	More effective in AA than in EUR ancestry for hypertension	Probably related to increase predisposition of AA to the salt sensitive form of essential hypertension	Cushman et al (121) Aviv (123)
VI-Hepatitis anti-viral treatment			
Ribavirin (Interferon α)	AA have lower rate of response to treatment than EUR ancestry	Due to differing immune ability as AA produces more cytokines than EUR	Muir et al (124) Kimball et al (125)
Drugs in which difference is replicated in numerous studies but no inference about physiological basis			
I-Vasodilator antihypertensive			
Sodium nitropruside	Attenuated response to multiple vasodilators in AA than EUR ancestry	Mechanism not fully under stood	Stein et al (126) Rosenbaum et al (127)
II-Glucocorticoids			
Methyl prednisolone	Adverse effect more common in AA than in EUR ancestry	Also altered pharmacokinetics between AA and EU	Tornatoro et al (128)
Ill-Anti-Diabetic			
Insulin	Insulin sensitivity significantly lower in Hispanics and African AA than in EUR	Differences remain after adjusting for body fat results well replicated	Goran et al (129)

EUR=Europeans; AA=African-America

consistent association of individual SNPs with bronchodilator responsiveness. Rather, complex promoter and coding region haplotypes containing multiple SNPs alter receptor expression and predict *in vivo* responsiveness. The β-1-adrenoreceptor variant (Arg 389) has been reported as associated with increased response to beta-blockers (105–106). Frequency of this variant varies significantly between European Americans –0.723 and African Americans –0.575.

As mentioned earlier, null allele of CYP2D6, a drug-metabolizing enzyme (DME) is reported in a frequency of 10% among north European ancestry and therefore they do not experience an analgesic effect from the prodrug codeine (95). On the contrary, about 98% Arabs are able to transform codeine into the active form morphine (107). CYP2D6 enzyme is a member of the hepatic cytochrome P450 family, and metabolizes 25–30% of all clinically used medications, including antidepressants, antipsychotics, β-blockers, antiarrhythmics and opioid analgesics. The CYP2D6 gene is the most variable of the P450 family, with over 75 different alleles. Variants arise from point mutations, single base-pair deletions and additions, deletion of the entire gene, and gene duplication resulting in two or more copies of the gene (95). The functional consequences include an increase, decrease or loss of enzyme activity that can be correlated with a change in *in vivo* function. Thus, extensive metabolizers (75-85% of the population) are homozygous or heterozygous for the wild-type enzyme or for variants with normal enzyme activity; intermediate metabolizers (10-15%) or poor metabolizers (5-10%) are carriers of two decreased-activities or loss-of-function alleles; and ultra-rapid metabolizers (1–10%) are carriers of duplicate or multiple active genes (95, 107).

Another important issue of using the information of genetic variation is to assess the adverse drug reactions. Use of an antiretroviral drug *'Abacavir'* to treat HIV infections develops a hypersensitivity reaction in about 5% of the people. It has been reported that people carrying HLA-B*5701 allele are associated with hypersensitivity to *'Abacavir'* among people of European American ancestry (108) and not among African Americans (109).

Importance of using the genetic information instead of proxy ethnic or racial ancestry was well documented in the trial of *'BilDil'* drug among African-Americans. *'BilDil'* is a combination drug that combines isosorbide diniterate (a nitric oxide donor) and hydralazine (a vasodilator agent) designed to restore low or depleted nitric oxide levels in the blood to treat or prevent cases of congenital heart failure (110). The trial was conducted due to inefficacy of BilDil in treating congestive heart failure among African-Americans in two ethnically mixed clinical trials (111–112). The latest trial has been recently halted because *'BilDil'* was found highly effective in treating all blacks (113).

Conclusion

Categorical reviewing of the studies carried out on different genetic variants laid emphasis on the fact that the genetic variation between different populations and individuals is a key factor behind pathogenesis of diseases as well as responses and side effect of drugs. However, this

consensus view appears too narrow because of the heterogeneity of results of pharmacognomics studies carried out at different centres on patients of different ethnic descent. This makes it difficult to identify the most potent genetic variant that can be used as a predictive marker for the success of a drug with some notable exception.

Despite the challenges of Pharmacogenomic research, the potential impact of revealing the genetic underpinnings of variable drug response is too significant to ignore and significant strides have already been made in using this new science. DNA diagnostic tests will become available to define a population of patients that are more likely to respond to a drug and be at less risk of a side effect or adverse reaction. Implementing the results of Pharmacogenomic research will revolutionize medicine. Such studies open a plethora of options that can bring into practice like timing and doses of drug therapies like that of immunosuppressive regimens in case of renal or bone marrow graft rejections or the supplementation of folic acids in neural tube defects or type of anti-hypertensive and anti-coagulatory therapy to overcome the cases of coronary heart diseases and thrombofilia. Incorporation of such study will allow an advance anticipation of clinical outcome and drug response and will cause a shift from 'One treatment fits all' approach.

ACKNOWLEDGEMENTS

Authors are thankful to Department of Biotechnology (DBT) New Delhi and Sanjay Gandhi Post Graduate Institute of Medical Sciences Lucknow.

REFERENCES

1. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG et al. The sequence of the human genome. *Science* 2001; 291: 1304–1351.
2. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J et al. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001: 409: 860–921.
3. Cavalli-Sforza LL, Feldman MW. The application of molecular genetic approaches to the study of human evolution. Nat Genet 2003; 33 Suppl: 266–275.
4. Collins FS, Green ED, Guttamacher AE and Guyer MS. A vision for the future genomics research. *Nature* 2003; 422: 835–847.
5. Tishkoff SA, Kidd KK. Implications of biogeography of human populations for 'race' and medicine. *Nat Genet* 2004; 36: S21–S27.
6. Tate SK, Goldstein DB. Will tomorrow's medicines work for everyone? *Nat Genet* 2004; 36: S34–S42.
7. Klein TE, Chang JT, Cho MK et al. Integrating genotype and phenotype information: an overview of the PharmGKB project. Pharmacogenetics Research Network and Knowledge Base. *Pharmacogenomics J* 2001; 1: 167–170.
8. Evans WE, McLeod H. Pharmacogenomics – Drug disposition, drug targets, and side effects. *N Eng J Med* 2003; 348: 538–549.
9. Reynolds GP, Templeman LA, Godlewska BR. Pharmacogenetics of schizophrenia. *Expert Opin Pharmacother* 2006; 7: 1429–1440.
10. Zineh I, Johnson JA. Pharmacogenetics of chronic cardiovascular drugs: applications and implications. *Expert Opin Pharmacother* 2006; 7: 1417–1427.
11. Roden DM, George AL. The genetic basis of variability in drug responses. *Nature Review Drug Disc* 2002; 11: 37–44.

12. Liggett SB. β_2-Adrenergic receptor pharmacogenetics. *Am J Respir Cnt Care Med* 2000; 161: S197–S201.

13. Weinshilboum R. Inheritance and drug response. *New England Journal of Medicine* 2003; 348: 529–537.

14. Vogel F. Moderne problem der humangenetik. *Ergeb Inn Med U Kinderheilk* 1959; 12: 52–125.

15. Vesell ES, Page JG. Genetic control of drug levels in man: phenylbutazone. *Science* 1968; 159: 1479–1480.

16. Ainoon O, Boo NY, Yu YH, Cheong SK, Hamidah HN. G6PD deficiency with hemolytic anemia due to a rare gene deletion-A report of the first case in Malaysia. *Hematology* 2006; 11: 113–118.

17. Couzin J. Pharmacogenomics: cancer sharpshooters rely on DNA tests for a better aim. *Science* 2004; 305: 1222a–1223a.

18. Savona M, Talpaz M. Chronic myeloid leukemia: changing the treatment paradigms. *Oncology* 2006; 20: 707-711.

19. Khan IA, Gowda RM. Novel therapeutics for treatment of long-QT syndrome and torsade de pointes. *Int J Cardiol* 2004; 95: 1–6.

20. Fischer A, Wiebe V, Paabo S, Przeworski M. Evidence for a complex demographic history of chimpanzees. *Mol Biol Evol* 2004; 21: 799–808.

21. Jorde LB, Wooding SP. Genetic variation, classification and 'race'. *Nat Genet* 2004; 36: S28–S33.

22. Khan F, Agrawal S, Agrawal F. Genetic predisposition and renal allograft failure: an implication on non-HLA genetic variants. *Mol Diagnosis Therapy* 2006: 10: 1–18.

23. McDaniel DO, Barber WH, Nguyan C, et al. Combined analysis of cytokine genotype polymorphism and the level of expression with allograft function in African-American renal transplant patients. *Trans Immunol* 2003; 11: 107–119.

24. Suthanthiran M. The importance of genetic polymorphisms in renal transplantation. *Curr Opin Urol* 2000; 10: 71–75.

25. Hutchinson IV, Pravica V, Perrey C, et al. Cytokine gene polymorphisms and relevance to forms of rejection. *Transplant Proc* 1999; 31: 734–736.

26. Hahn AB, Kasten-Jolly JC, Constantino DM, et al. TNF-alpha, EL-6, IFN-gamma, and IL-10 gene expression polymorphisms and the IL-4 receptor alpha-chain variant Q576R: effects on renal allograft outcome. *Transplantation* 2001; 72: 660-665.

27. Kruger B, Schroppel B, Ashkan R, et al. A Monocyte chemoattractant protein-1 (MCP-1) polymorphism and outcome after renal transplantation. *J Am Soc Nephrol* 2002; 13: 2585–2589.

28. Enard W, Przeworski M, Fisher SE et al. Molecular evolution of FOXP2, a gene involved in speech and language. *Nature* 2002; 418: 869–872.

29. Verrelli BC, McDonald JH, Argyropoulos G et al. Evidence for balancing selection from nucleotide sequence analyses of human G6PD. *Am J Hum Genet* 2002; 71: 1112–1128.

30. Sabeti PC, Reich DE, Higgins JM et al. Detecting recent positive selection in the human genome from haplotype structure. *Nature* 2002; 419: 832–837.

31. Hamblin MT, Thompson EE, Di Rienzo A. Complex signatures of natural selection at the Duffy blood group locus. *Am J Hum Genet* 2002; 70: 369–383.

32. Wooding SP, Watkins WS, Bamshad MJ et al. DNA sequence variation in a 3.7-kb non-coding sequence 5' of the CYP1A2 gene: implications for human population history and natural selection. *Am J Hum Genet* 2002; 71: 528–542.

33. Osier M, Pakstis AJ, Kidd JR et al. Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism. *Am J Hum Genet* 1999; 64: 1147–1157.

34. Rotimi CN. Are medical and nonmedical uses of large-scale genomic markers conflating genetics and 'race'? *Nat Genet* 2004; 36: S43-S47.

35. Braun L. Race, ethnicity and health: can genetics explain disparity? *Perspect Biol Med* 2002; 45: 159–174.

36. Bertsch B, Ogden CA, Sidhu K, Le-Niculescu H, Kuczenski R, Niculescu AB. Convergent functional genomics: a Bayesian candidate gene identification approach for complex disorders. *Methods* 2005; 37: 274–279.

37. Weiss ST, Lake SL, Silverman ES, et al. Asthma steroid pharmacogenetics: a study strategy to identify replicated treatment responses. *Proc Am Thorac Soc* 2004; 1: 364–367.

38. Lee D, Weinshilboum RM. *Drug Metab Disgos* 1995; 23: 398–405.

39. Nasedkina TV, Fedorova OE, Glotov AS, et al. Rapid genotyping of common deficient thiopurine S-methyltransferase alleles using the DNA-microchip technique. *Eur J Hum Genet* 2006 (Under publication).

40. Lassiter SJ, Stryjewski W, Legendre BL Jr, et al. Time-resolved fluorescence imaging of slab gels for lifetime base-calling in DNA sequencing applications. *Anal Chem* 2000; 72: 5373–5382.

41. Underbill PA, Passarino G, Lin AA et al. The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. *Ann Hum Genet* 2001; 65: 43–62.

42. Oberacher H, Niederstatter H, Casetta B, Parson W. Detection of DNA sequence variations in homo- and heterozygous samples via molecular mass measurements by electrospray ionization time-of-flight mass spectrometry. *Anal Chem* 2005; 77: 4999–5008.

43. Patil N, Berno AJ, Hinds DA, et al. Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* 2001; 294: 1719–1723.

44. Chakravart1 A. Single nucleotide polymorphism.... to a future of genetic medicine. *Nature* 2001: 409: 822–823.

45. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 1994; 265: 2037–2048.

46. Risch NJ. Searching for the genetic variants in the new millennium. *Nature* 2000; 405: 847–856.

47. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003; 33: 177–182.

48. Cardon LR, Bell JI. Association study designs for complex diseases. *Nat Rev Genet* 2001; 2: 91–99.

49. Altshuler D, Kruglyak L, Lander E. Genetic polymorphisms and disease. *N Engl J Med* 1998; 338: 1626.

50. Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999; 65: 220–228.

51. Hinds DA, Stokowski RP, Patil N, et al. Matching strategies for genetic association studies in structured populations. *Am J Hum Genet* 2004; 74: 317–325.

52. Knowler WC, Williams RC, Petitt RC and Steinberg AG. Gm and type II diabetes mellitus; an association in American Indians with genetic admixture. *Am J hum Genet* 1988; 43: 52–526.

53. Rosenberg NA, Pritchard JK, Weber JL et al. Genetic structure of human populations. *Science* 2002; 298: 2381–2385.

54. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000; 155: 945–959.

55. Risch N, Tang H, Katzenstein H and Ekstein J. Geographic distribution of disease mutations in Ashkenazi Jewish population supports genetic drift over selection. *Am J Hum Genet* 2003; 72: 812–822.

56. Pereira AC, Mota GA, Bensenor I, Lotufo PA, and Krieger JE. Effect of race, genetic population structure, and genetic models in two-locus association studies: clustering of functional renin-angiotensin system gene variants in hypertension association studies. *Braz J of Med and Biol Res* 2001; 34: 1421–1428.

57. Bamshad M, Kivisild T, Watkins WS et al. Genetic evidence on the origins of Indian caste populations. *Genome Res* 2001; 11: 994–1004.

58. Cordaux R, R Aunger, G Bentley et al. Independent origins of Indian caste and tribal paternal lineages. *Curr Biol* 2004: 14: 231–235.

59. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet* 1995; 346: 1133–1134.

60. Gupta N, Khan F, Tripathi M et al. Absence of factor V Leiden (G1691A) mutation, FII G20210A allele in coronary artery disease in North India. *Indian J Med Sci* 2003; 57: 535–542.

61. Mayer B, Schunkert H. ACE gene polymorphism and cardiovascular disease. *Herz* 2000; 25: 1–6.

62. Agrawal S, Singh VP, Tewari S. Angiotensin-converting enzyme gene polymorphism in coronary artery disease in north India. *Indian Heart J* 2004; 56: 44–46.

63. Tripathi G, Dharmani P, Khan F, Kumar V, Sharma RK, Agrawal S. High prevalence of ACE DD genotype among end stage renal disease (ESRD) patients from north India. *BMC Nephrology* 2006 (Under publication).

64. Fodinger M, Wolfl G, Fischer G, et al. Effect of MTHFR 677C>T on plasma total homocysteine

levels in renal graft recipients. *Kidney Int* 1999; 55: 1072–1080.

65. Viklicky O, Hubacek JA, Kvasnicka J, et al. Association of methylene tetra hydro folate reductase T677 allele with early development of chronic allograft nephropathy. *Clin Biochem* 2004; 37: 919–247.
66. Fischereder M, Gohring P, Schneeberger H, et al. Early loss of renal transplants in patients with thrombophilia. *Transplantation* 1998; 65: 936–939.
67. Irish AB, Green FR, Gray DW, Morris PJ. The factor V Leiden (R506Q) mutation and risk of thrombosis in renal transplant recipients. *Transplantation* 1997; 64: 604–607.
68. Anglicheau D, Verstuyft C, Laurent-Puig P, et al. Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nerphrol* 2003; 14: 1889–1896.
69. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci USA* 2000; 97: 3473–3478.
70. Hitzl M, Drescher S, van der Kuip H, et al.. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001; 11: 293–298.
71. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189–199.
72. Bakr AM, El-Chenawi F, Al-Husseni F. HLA alleles in frequently relapses steroid-dependent and -resistant nephritic syndrome in Egyptian children. *Pediatr Nephrol* 2005; 20: 159–162.
73. Turner ST, Schwartz GL. Gene markers and antihypertensive therapy. *Curr Hypertens Rep* 2005; 7: 21–30.
74. Emilien G, Ponchon M, Caldas C, Isacson O, Maloteaux JM. Impact of genomics on drug discovery and clinical medicine. *QJM* 2000; 93: 391–423.
75. Vernon SD, Reeves WC. The challenge of integrating disparate high-content data: epidemiological, clinical and laboratory data collected during an in-hospital study of chronic fatigue syndrome. *Pharmacogenomics* 2006; 7: 345–354.
76. Motsinger AA, Ritchie MD. Multifactor dimensionality reduction: an analysis strategy for modelling and detecting gene-gene interactions in human genetics and pharmacogenomics studies. *Hum Genomics* 2006; 2: 318–328.
77. Yi J, Zhang W, Zhang DY. Molecular Zipper: a fluorescent probe for real-time isothermal DNA amplification. *Nucleic Acids Res* 2006; 34: e81.
78. Li F, Zhao C, Zhang W, et al. Use of ramification amplification assay for detection of Escherichia coli O157:H7 and other E. coli Shiga toxin-producing strains. *J Clin Microbiol* 2005; 43: 6086–6090.
79. Egashira T. [Development and clinical application of invader assay—detection of resistant mutation to clarithromycin in Helicobacter pylori] Rinsho Byori 2006; 54: 176–183.
80. Germer JJ, Majewski DW, Yung B, Mitchell PS, Yao JD. Evaluation of the invader assay for genotyping hepatitis C virus, *J Clin Microbiol* 2006; 44: 318–323.
81. Dummitt B, Chang YH. Molecular beacons for DNA binding proteins, an emerging technology for detection of DNA binding proteins and their ligands. *Assay Drug Dev Technol* 2006; 4: 343–349.
82. Wyatt MF, Stein BK, Brenton AG. Investigation into accurate mass capability of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, with respect to radical ion species. *J Am Soc Mass Spectrom* 2006; 17: 672–675.
83. Klenkar G, Valiokas R, Lundstrom I, et al. Piezo dispensed microarray of multivalent chelating thiols for dissecting complex protein-protein interactions. *Anal Chem* 2006; 78: 3643–3650.
84. Jain KK. Applications of biochip and microarray systems in pharmacogenomics. *Pharmacogenomics* 2000; 1: 289–307.
85. Keller MA. Molecular diagnostic testing for inherited thrombophilia using Invader. *Methods Mol Med* 2005; 114: 107–119.
86. Molnar S. Human variation: Races, Types and Ethic Groups 5th edn. (Prentice hall, New Jersey, 2001).

87. Luzzatto L, Mehta A, Meloni T. Haemoglobinuria and haptoglobin in G6PD deficiency. *Br J Haematol* 1995; 91: 511–512.

88. Tishkoff SA, Varkonyi R, Cahinhinan N et al. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science* 2001; 293: 455.

89. Rotimi CN. Are medical and nonmedical uses of large-scale genomic markers conflating genetics and 'race'? *Nat Genet* 2004; 36: S43-S47.

90. Kevles DJ. In the name of eugenics: Genetics and the uses of human heredity (Harvard University Press, Cambridge, 1995).

91. Bamshad MJ, Wooding S, Watkins WS et al. Human population genetic structure and inference of group membership. *Am J Hum Genet* 2003; 72: 578–589.

92. Reich JM. Course and prognosis of sarcoidosis in African-Americans versus Caucasians. *Eur Respir J* 2001; 17: 833.

93. Nakajima T, Wooding S, Sakagami T et al. Natural selection and population history in the human angiotensinogen gene (AGT): 736 complete AGT sequences in chromosomes from around the world. *Am J Hum Genet* 2004; 74: 898–916.

94. Kunz R, Kreutz R, Beige J, Distler A, Sharma AM. Association between the angiotensinogen 235T-variant and essential hypertension in whites: a systematic review and methodological appraisal. *Hypertension* 1997; 30: 1331–1337.

95. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002; 3: 229–243.

96. Pritchard JK, Cox NJ. The allelic architecture of human disease gengs,: common disease-common variant ... or not? *Hum Mol Genet* 2002; 11: 2417–2423.

97. Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. *Genome Res* 1998; 8: 1229–1231.

98. Bamshad M, Wooding S, Salisbury BA, Stephens JC. Deconstructing the relationship between genetics and race. *Nat Rev Genet* 2004; 5: 598–609.

99. Martin MP, Dean M, Smith MW et al. Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science* 1998; 282: 1907–1911.

100. Gonzalez E, Bamshad M, Sato N et al. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. *Proc Natl Acad Sci USA* 1999; 96: 12004–12009.

101. Bamshad MJ, Mummidi S, Gonzalez E et al. A strong signature of balancing selection in the 5' cis-regulatory region of CCR5. *Proc Natl Acad Sci USA* 2002; 99: 10539–10544.

102. Ogura Y, Bonen DK, Inohara N et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; 411: 603–606.

103. Hugot JP, Chamaillard M, Zouali H et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411: 599–603.

104. Inoue N, Tamura K, Kinouchi Y et al. Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 2002; 123: 86–91.

105. Sofowora GG, Dishy V, Muszkat M et al. A common betal-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to beta-blockade. *Pharmacol Ther* 2003; 73: 366–371.

106. Johnson JA, Zineh I, Puckett BJ et al. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. *Clin Pharmacol Ther* 2003; 74: 44–52.

107. McLellan RA, Oscarson M, Seidegard J, Evans DA, Ingelman-Sundberg M. Frequent occurrence of CYP2D6 gene duplication in Saudi Arabians. *Pharmacogenetics* 1997; 7: 187–191.

108. Hetherington S, Hughes AR, Mosteller M et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002; 359: 1121–1122.

109. Mallal S, Nolan D, Witt C et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002; 359: 727–732.

110. Taylor AL, Cohn JN, Worcel M, Franciosa JA and A-HeFt investigators. The African-American heart failure trial: background, rationale and significance. *J Natl Med Assoc* 2002; 94: 762–769.

111. Cohn JN, Archibald DG, Ziesche S et al. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans

Administration Cooperative Study. *N Eng J Med* 1986; 314: 1547–1552.

112. Cohn JN, Johnson G, Ziesche S et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. *N Eng J Med* 1991; 325; 303–310.

113. Pollack A. Drug approved for heart failure in black patients. *New York Times* 2004.

114. Cubeddu LX, Aranda J, Singh B et al. A comparison of verapamil and propranolol for the initial treatment of hypertension. Racial differences in response. *JAMA* 1986; 256: 2214–2221.

115. Friedman B, Gray JM, Gross S, Levit SA. United States experience with oxprenolol in hypertension. *Am J Cardiol* 1983; 52: 43D–48D.

116. Kallinowski L, Dobrucki IT and Malinski T. Race specific differences in endothelial function: predisposition to African-Americans to vascular diseases. *Circulation* 2004; 109: 2511–2517.

117. Exner DV, Dries DL, Domanski MJ, Cohn JN. Lesser response to angiotensin-converting-enzyme inhibitor therapy in black as compared with white patients with left ventricular dysfunction. *N Eng J Med* 2001; 344: 1351–1357.

118. Weir MR, Reisin E, Falkner B et al. Nocturnal reduction of blood pressure and the antihypertensive response to a diuretic or angiotensin converting enzyme inhibitor in obese hypertensive patients. TROPHY Study Group. *Am J Hypertens* 1998; 11: 914–920.

119. Weir MR, Gray JM, Paster R, Saunders E. Differing mechanisms of action of angiotensin-converting enzyme inhibition in black and white hypertensive patients. The Trandolapril Multi-center Study Group. *Hypertension* 1995; 26: 124–130.

120. Carson P, Ziesche S, Johnson G, Cohn JN. Racial differences in response to therapy for heart failure: analysis of the vasodilator-heart failure trials. Vasodilator-Heart Failure Trial Study Group. *J Card Fail* 1999; 5: 178–187.

121. Cushman WC, Reda DJ, Perry HM et al. Regional and racial differences in response to antihypertensive medication use in a randomized controlled trial of men with hypertension in the United States. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. *Arch Intern Med* 2000; 160: 825–831.

122. Chapman AB, Schwartz GL, Boerwinkle E, Turner ST. Predictors of antihypertensive response to a standard dose of hydrochlorothiazide for essential hypertension. *Kidney Int* 2002; 61: 1047–1055.

123. Aviv A. Cellular calcium and sodium regulation, salt-sensitivity and essential hypertension in African Americans. *Ethn Health* 1996; 1: 275–281.

124. Muir AJ, Bornstein JD, Killenberg PG; Atlantic Coast Hepatitis Treatment Group. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N Eng J Med* 2004; 350: 2265–2271.

125. Kimball P, Elswick RK, Shiffman M. Ethnicity and cytokine production gauge response of patients with hepatitis C to interferon-alpha therapy. *J Med Virol* 2001; 65: 510–516.

126. Stein CM, Lang CC, Nelson R, Brown M, Wood AJ. Vasodilation in black Americans: attenuated nitric oxide-mediated responses. *Clin Pharmacol Ther* 1997; 62: 436–443.

127. Rosenbaum DA, Pretorius M, Gainer JV et al. Ethnicity affects vasodilation, but not endothelial tissue plasminogen activator release, in response to bradykinin. *Arterioscler Thromb Vase Biol* 2002; 22: 1023–1028.

128. Tornatore KM, Biocevich DM, Reed K et al. Methylprednisolone pharmacokinetics, cortisol response, and adverse effects in black and white renal transplant recipients. *Transplantation* 1995; 59: 729–736.

129. Goran MI, Bergman RN, Cruz ML and Watnabe R. Insulin resistance and associated compensatory responses in African American and Hispanic children. *Diabetes Care* 2002; 25: 2184–2190.