CLINICAL TESTING OF GENETIC VARIATION THAT GIVES RISE TO DIFFERING RESPONSE TO DRUGS

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Abstract:
The effect of variation in genes coding for drug targets and for the enzymes involved in drug metabolism has highlighted the genetic component of drug response. Drug response can be likened to a complex, multifactorial genetic trait, and the study of its genetic variation, termed pharmgenetics, is analogous to the study of complex genetic disease in terms of the questions posed and the analytical possibilities. A single human genome was a monumental effort a decade ago, more than one thousand genomes have now been sequenced. The task ahead lies in transforming this information into personalized treatment strategies that are tailored to the unique genetics of each individual. One important aspect of personalized medicine is patient-to-patient variation in drug response. Pharmacogenomics addresses this issue by seeking to identify genetic contributors to human variation in drug efficacy and toxicityGenetic approaches used to analyze rare and common adverse effects as well as variability in efficacy are first presented. The challenges and potential solutions to incorporating this body of knowledge into contemporary medical practice are then discussed.

1.0 Introduction:

A primary aim of pharmacogenomics has been to uncover novel human genetic variants that affect therapeutic response phenotypes and to identify the genes responsible for those phenotypic differences. The ultimate goal of the field has been to use an understanding of these relationships to devise novel personalized pharmacological treatment strategies that maximize the potential for therapeutic benefit and minimize the risk of adverse side effects for any given medication. The potential cost savings (via increased drug efficacy) and decreased morbidity and mortality (via increased drug safety) is immense. Advances in DNA sequencing and polymorphism characterization technologies have driven the field from a hypothesis-driven approach to a discovery-oriented, genome-wide approach that requires few assumptions regarding which variants are most relevant for outcome. Candidate gene approaches resulted primarily in the identification of genetic variants in drug metabolizing genes with large effects on toxicity or response however, many genome-wide association studies (GWAS) have identified novel associations between drug response and genetic variants with unknown functional relevance and often with relatively small effect sizes The recent development of high-throughput sequencing techniques has allowed researchers to begin to examine the contribution of rare variants to drug sensitivity Although many important discoveries have been made,

several challenges remain before the dream of personalized medicine will be realized. First, we must move from collecting large numbers of identified genetic variants to systematically analyzing them. Second, we must find ways to turn this systematic biological understanding into clinical strategies for treatment. The aim of this review is to provide an overview of recent exciting trends towards meeting these challenges.

Heritability and drug responses:

Studies in families can define the extent to which common human disease phenotypes like myocardial infarction or sudden cardiac death include a heritable component. However, it is usually not possible to accumulate well-defined drug response phenotypes across multiple related patients with the same disease; as a result, the heritable component of variability in drug action may not be well-defined. An in vitroapproach that has been useful to estimate heritability of cytotoxicity due to anticancer agents is exposure of lymphoblastic cell lines from related subjects to the drug Using this method, the heritability of cytotoxicity has been estimated at 0.25–0.65; one study went on to use linkage analysis to identify a potential locus mediating this toxicity.One approach when heritability is not well-understood is to quantify drug responses in multiple healthy members of a family. For example, very early studies in twins demonstrated far more variability in the urinary excretion of isoniazid within dizygotic than monozygotic twins, thus establishing that this trait – now known to reflect genetically-determined variable N-acetylation – is heritable.

2.0 Literature review:

Merry clark AT cadet (20003) The most common form of DNA variation in the human genome is the Single Nucleotide Polymorphism (SNP) SNP is a result of a mutation in which a single nucleotide is substituted by another nucleotide at a given position. SNPs occur once every 300-3,000 base-pairs if one compares the genomes of two unrelated individuals On an average it can be said that one SNP occurs in the sequence of 1000 base-pairs. Thus in the entire genome the estimate of the SNPs would be around 3.2 million in 3.2 billion nucleotides. SNPs are responsible for most of medically important SNPs/traits that are related to the of the predisposition individuals to certain diseases and differential drug response

Bakker E., Roos RA.(2010)the extent of polymorphism in the genes encoding enzymes that metabolize drugs and other xenobiotic is more compared to other genes. Drug response is better correlated with polymorphism in these genes encoding enzymes. Hence it is important to study the genes responsible for drug metabolism. Several common genetic polymorphisms had been identified on a phenotypic basis prior to the characterization of relevant genes. The DNA samples from individuals of known phenotype after sequence analysis, led to the identification of the polymorphisms responsible for the phenotype, and the development of reliable genotyping assays based mainly on the polymerase chain reaction

Hosford DA., Riley JH., et al.(2009) The incorporation of genetic information in this fashion into EMRs could also enable a future vision in which drug outcomes can be not only queried retrospectively but also followed prospectively, to generate

new genotype-drug response relationships. Francis Collins enunciated this vision after being appointed NIH director, when he said: "The limiting factor right now is thatoften times, if you are ready to write a prescription, you donot want to wait a week to find out the genotype before you decide whether you've got the right dose and the right drug But if everybody's DNA sequence is already in their medical record and it is simply a click of the mouse to find out allthe information you need, then there is going to be a much lower barrier to beginning to incorporate that information into drug prescribing.

3.0 Experimental approaches in pharmacy genetics:

Defining mechanisms underlying variable drug concentrations and effects provides a starting point for identifying candidate genes for further pharmcongeneric study. As a result, many important examples in pharmcogenesis relate to variable drug uptake, metabolism, or elimination. Other contributors to variable drug responses identified by this physiologically-based candidate approach include variation in drug target molecules or in disease pathways. In some cases, variants in multiple genes have been implicated, as discussed below More recently, technologies to search for previously unanticipated relationships between phenotypes and hundreds of thousands of common polymorphic sites across the genome (an unbiased approach) have been applied to the problem of variable drug actions; these genome-wide association studies (GWAS) have been conducted both in human cohorts as well as in cellular or model organ systems. Table lists the experimental approaches that have been used in the field, along with their potential advantages and disadvantages.Replication of genotype-phenotype relations can be a major issue in modern genomics, both when the effects of single candidate variants are examined as well as with genome-wide approaches Pharmacygenetic studies may be especially difficult to replicate because large numbers of subjects with well-curated drug response phenotypes are often not available.

Model organisms and mechanisms:

In addition to the cell-based approaches mentioned above (immortalized lymphoblastic cell lines), unbiased studies in model organisms have also been used to identify contributions of loci across the genome to drug response phenotypes. Milan et al. demonstrated that QT prolonging drugs reproducibly produce Bradycardiac and atriaventricular block in zebra fish embryos Challenging wild-type of mutagenized fish with dofeticide, a prototypical QT prolonging agent, identified multiple loci modulating this drug response phenotype.Interestingly, one of these, *GINS3*, also was implicated as a modulator of the normal QT duration in GWAS analyses of tens of thousands of subjects An example of how unbiased discovery approaches can lead to new understanding new mechanisms is the finding that a SNP in the HMG-CoA reeducates gene, initially identified as a modulator of response to inhibitor drugs, generates an alternatively spliced mRNA that encodes a protein with reduced drug sensitivity

Table: Approaches to identifying and validating genetic influences on drug response

Approac	Adva	Disadv	Examples

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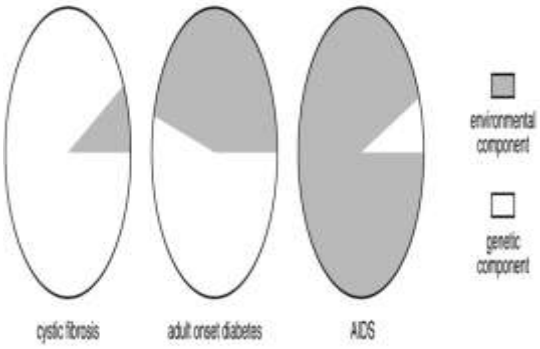
Candidate gene based on variable pharmacokinetics	Variability in these processes logically determine variable drug effects Candidate genes often identified	Identification and replication of associations between variant genotypes and drug responses may require large populations, depending on the size of the genetic effect and the frequency of the variant allele	Warfarin/CYP2C9 Simvastatin/SLCO1B1 Clopidogrel/CYP2C19[1][1] Metoprolol/CYP2D6 Atorvastatin/CYP3A5
Candidate gene based on variable pharmacodynamics			Bucindolol/ADRB1 Beta-blockers in heart failure/ACE Antiarrhythmics in atrial fibrillation/ACE Warfarin/VKORC1
Candidate pathway analysis	Possibly less biased than single gene approaches	Requires interrogation of large numbers of SNPs; replication may be difficult	HMG-CoA reductase haplotype as a predictor of statin response

Problem of genetic heterogeneity:

Even from these single-gene disorders, we have indications that the situation is not that simple. Geneticists are familiar with terms such as "epigenetics," ie, genetic phenomena that cannot be explained by traditional genetics.One example of this is disease transmission and severity being affected by the sex of the transmitting parent and "modifying factors" (probably other genes that affect, disease severity); this is the case for cystic fibrosisand hemochromatosis. We increasingly observe that, even if the gene causing the disorder is known, the phenotype-genotype relationship is not clear.This genetic heterogeneity poses a real problem for diagnostic testing. The connection between a patient's symptoms, diagnosis, and the underlying mechanism of disease is often obscure. For example, patients with mutations in different, genes may present as clinically identical, while patients with the same mutation may present, clinically disparate.



DNA arrays and chip technology

Most, of the hope is placed on DNA arrays and chip technology, which have been developed over the last 5 years. High-density DNA arrays allow complex mixtures of RNA and DNA to be interrogated in a parallel and quantitative fashion. While the making of arrays with more than several hundred elements was until recently a significant technical

achievement, arrays with more than 250 000 different oligonucleotide probes or 10 000 different cDNAs (transcribed DNAs) per square centimeter can now be produced in significant, numbers. DNA chips are simply glass surfaces bearing arrays of DNA fragments at discrete addresses, at which the fragments are available for hybridization. There are many variations on the chip theme, but the general approach is as follows:

- To immobilize multiple DNA samples on a solid support, which are then interrogated (hybridized) with a pool of oligonucleotide probes (a selected single stranded string of nucleotides) that are specific for particular mutations or DNA variants (allele-specific oligonucleotides, ASOs).

4.0 Genetic variation and current testing for monogenic disorders

The clinical use of genetic variation in the evaluation of cancer risk is expanding, and thus understanding how determinants of cancer susceptibility identified in one population can be applied to another is of growing importance. However there is considerable debate on the relevance of ethnic background in clinical genetics, reflecting both the significance and complexity of genetic heritageIt has been well known for many years that DNA sequence is highly variable, even within populations. DNA variation can be in the form of single nucleotide substitutions, the deletion or insertion of one or more nucleotides, or the variable repetition of a number of nucleotides (small tandem repeats [STRs] or longer variable number of tandem repeats [VNTRs]). Neutral DNA changes or "variants" (with respect to selective pressures) are referred to as polymorphisms when their rarest allele is present, in more than 1 % of chromosomes in a particular population. Mutations, on the other hand, are rare differences that occur in less than 1 % of the population (usually much less than 1%) and have typically been discovered in the coding sequences of genes causing rare inherited diseases. How neutral the so-called polymorphisms really arc is merely assumed on the basis of their lack of direct association with a particular phenotype. However, it is feasible to assume that a particular variant may produce a particular phenotype when in combination with particular alleles of other such variants.The ability to screen particular genes for mutations has developed into an important diagnostic tool, and genetic testing for disorders that, are inherited in a Mendelian fashion (primarily single-gene disorders, so-called monogenic) is already well established in medical practice. This is relatively easily performed for monogenic disorders when the causative gene is known, eg, cystic fibrosis, hemophilia, various forms of muscular dystrophy, mental retardation, and late-onset neurological disorders. Testing for mutations in specific disease genes can help diagnose the disease, determine the carrier status of an individual, and predict the occurrence of the disease.

Genetic testing in the future: new technology:

There is a general tendency in human genetics to move away from studies of single genes to genome-wide approaches. The genetic testing for inherited disorders is following the same trend and, similarly, the emphasis in testing for drug response will move from the analysis of single genes affecting drug metabolism, to the large-scale analysis of genetic variation in

relation to drug response. Instead of investigating polymorphisms close to candidate genes, thousands of variants (SNPs) across the genome will be typed and organized into an individual "fingerprint," also referred to as an SNP printor, for the sake of this review, an SNP pharmgenetic profile. he allele frequencies of DNA polymorphisms such as SNPs are highly variable between populations, so that population admixture may mask, blur, or alter the LD patterns Secondly, ethnic variation in drug response is well known: in World War II it was discovered that. African-American soldiers who were treated with the antimalarial drug primaquine developed hemolytic anemia crises at high altitudes, due to glucose-6-phosphate dehydrogenase deficiency. Hence, different SNP profiles relating to drug response can be expected in different populations. This concept, is not new to genetic testing, for example, mutation analysis for cystic fibrosis is already tailored to patients with different, ethnic backgrounds

Human Genetic Variation:

Two research approaches were historically important in helping investigators understand the biological basis of heredity. The first of these approaches, transmission genetics, involved crossing organisms and studying the off springs' traits to develop hypotheses about the mechanisms of inheritance As important as they were, the techniques of transmission genetics and cytology were not enough to help scientists understand human genetic variation at the level of detail that is now possible. The central advantage that today's molecular techniques offer is that they allow researchers to study DNA directly. Before the development of these techniques, scientists studying human genetic variation were forced to make inferences about molecular differences from the phenotypes produced by mutant genes. Furthermore, because the genes associated with most single-gene disorders are relatively rare, they could be studied in only a small number of families. Many of the traits associated with these genes also are recessive and so could not be detected in people with heterozygous genotypes.

Genetic test evaluation: The critical first step in genetic test evaluation is to precisely define the exact genetic variants that it is intended to assay, the disorder of interest, the purpose of the test, and the population or healthcare setting in which it is going to be used. Without such express specification, the evaluation will produce results of limited value.

Analytical validity

The analytical validity of a genetic test refers to the *assay* and defines its ability to measure accurately and reliably the genotype of interest. This part of the evaluation is concerned with assessing test performance in the laboratory as opposed to the clinic. Explicit specification of the genotype of interest is needed because the estimation of analytic validity is both method and mutation specific. The key quantative measures of assay performance for analytical validity are analytical sensitivity and specificity. With DNA-based technologies it is possible to achieve analytical sensitivity and specificity close to 100%.Quality assurance aims to ensure that test results are reliable and reproducible and usually include internal and external control assessments within a quality management framework. A recent survey on quality assurance of genetic

testing services in the European Union revealed that the participation of laboratories in external quality assessment schemes was fragmented and incomplete. Many laboratories did not have any accreditation.[14] These results suggest that the analytical validity of a significant proportion of the genetic tests currently provided by molecular laboratories within the European Union cannot be assured.

Clinical validity

Clinical validity defines the ability of a genetic test to detect or predict the presence or absence of the phenotype or clinical disease. Genetic association and other scientific studies may demonstrate a clear association between the presence of certain genetic variants and the disease, but will in itself not be sufficient to serve as a demonstration of clinical validity. he key causative mutations for a particular disorder can also vary between different populations. For example, studies of the clinical sensitivity of the American College of Medical Genetics panel of 25 mutations for cystic fibrosis has estimated that the clinical sensitivity of the panel was 71.9% for non- Hispanic Caucasians, 41.6% for African Americans and only 23.4% for Asian Americans.[15] The clinical sensitivity of this test was limited by the mutations chosen to be included in the panel for testing. It highlights the importance of knowledge of the frequency of specific genetic variants in a defined population

Conclusion:

Technical advances now allow faster and more reliable diagnostic capabilities and in the future will make more extensive screening programs a feasible option in both practical and economic terms. Despite the rapid pace of change in medical science, it seems certain that the identification of DNA alterations will remain a central part of medicine in the future. Diagnostics and pharmacogenomics are destined to a communal future. It is important that systems and infrastructure for genetic test evaluation are implemented. Although the provision of genetic tests for rare inherited diseases is of the greatest importance, the use of new molecular diagnostics for the common complex diseases such as cancer, coronary heart disease and diabetes may in time have significantly greater impact on population health. The complexities associated with the interpretation of tests for these common disorders and the technologies involved, will be far greater than for genetic tests used in the diagnosis of high penetrance monogenic disorders.

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