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Human Genetic Variation in Response to Medical and Environmental Agents: Pharmacogenetics and Ecogenetics

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Human Genetic Variation in Response to Medical and Environmental Agents: Pharmacogenetics and Ecogenetics

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Pharmacogenetics and Ecogenetics

The Problem and Its Scope

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Pharmacogenetics started in the mid-1950's with the demonstration that two unrelated drug reactions were caused by different genetically determined biochemical aberrations: pseudocholinesterase variation as the cause of succinylcholine sensitivity and abnormalities in red cell glutathione metabolism as an explanation for primaquine sensitivity. Genetic differences in acetylation of INH were shown soon thereafter. Impressed by these developments, I pointed out the relevance of genetic variation in the elucidation of drug reactions and Vogel coined the term pharmacogenetics (see Motulsky, 1972, for references).

The concept became fairly popular and fitted well with simultaneously developing insights into the ubiquity of biochemical polymorphisms. However, despite the discovery of many polymorphisms, the number of newly discovered monogenic pharmacogenetic traits remained modest. The field underwent a renaissance in the 1970's when Vesell and his collaborators demonstrated by drug metabolism studies that identical twins were more alike than nonidentical twins for many drugs tested (Vesell, 1973). Multifactorial inheritance was postulated with the implication that multiple unknown genetic determinants affected drug disposal.

The development of potent psychopharmacologic agents, which exhibited considerable variation in their effects, directed attention to the role of possible genetic factors in their action. Together with renewed interest in behavioral genetics, an area of pharmacogenetics known as psychopharmacogenetics (Omenn and Motulsky, 1976) was defined and deals with genetic factors in this general area. Considerations of alcohol metabolism are part of this field.

The development of blood level assays of drugs is accelerating and used with increasing frequency in medicine. The wide variability in blood levels of many drugs is now being observed by many physicians. Individualization of therapy by use of drug level assays is practiced and new data are being produced. Study designs and analysis of blood levels of drugs using genetic concepts and techniques are lagging since clinical pharmacologists who are doing such work usually lack a

background in genetics. The twin studies suggest that those rare individuals representing the extremes of the normal distribution curve (with low and high blood levels, respectively) owe their deviant response largely to genetic variability. It can be concluded that genetic factors are an important explanation for variability in response to most drugs. While variable drug metabolism will often be responsible for this variability, other mechanisms may also be operative. Pharmacogenetics has moved from a field that dealt with a few unusual drug reactions to a discipline of central importance for pharmacology and therapeutics.

Considerable advances in pharmacokinetics have also been made within the last 10 years. From the hobby of a few pioneers (e.g., Dost, 1953) the field has become an integral portion of clinical pharmacology. The availability of computers allows the handling of large bodies of data using different kinetic models. The introduction of genetic concepts and experimental designs in studies of pharmacokinetics promises to yield many new and interesting data. Some early attempts have been made in some recent twin studies on salicylate metabolism (Furst et al., 1977). The field is wide open for additional work.

Brewer (1971) in an editorial in the *American Journal of Human Genetics* in 1971 first coined the term 'ecogenetics'. He generalized that genetic variation not only was relevant to drug action but needed to be considered in responses to any kind of environmental agent. The concept was further elaborated by Omenn and myself in several recent publications (Omenn and Motulsky, 1978; Motulsky, 1977). There has been much recent public concern with problems of teratogenesis, mutagenesis, and carcinogenesis. For lack of simple approaches, few studies of human variability in response to xenobiotic agents (agents foreign to normal metabolism with potential biologic effects) have been done. Species differences in xenobiotic metabolism make it unlikely that lower animals can be used to predict teratogenesis, mutagenesis, and carcinogenesis consistently in man. Arguing from pharmacogenetic phenomena in man, it is likely that there is considerable variation in human metabolism of xenobiotic substances. If there is a dose-response relationship, as is likely for most agents, those human beings who are slow metabolizers for genetic reasons are at higher risk for mutations and cancer than those who metabolize foreign substances more rapidly. In other instances, some individuals may possess enzymes that activate inert compounds into carcinogenic substances. This aspect of the new pharmacogenetics appears to be of key importance for future studies of environmental mutagenesis and carcinogenesis, and needs to be stimulated and encouraged (Motulsky, 1977).

A more broadly oriented ecogenetics includes nutrition and the problems posed by genetic variation in response to foods. Topics such as hypolactasia, differences in iron absorption, and dietary problems posed by the genetic hyperlipidemias will be discussed at this conference. Other topics such as nitrate-induced headaches and tyramine-induced migraine are discussed elsewhere (Omenn and Motulsky, 1978). Wider aspects of ecogenetics such as interaction of climates and altitude with various human genotypes and human differences in susceptibility and resistance to microorganisms and to antigenic agents have also been summarized (Omenn and Motulsky, 1978).

The Future

Future studies in man, rather than in other species, remain essential. Imaginative use of blood and urine metabolites and more utilization of human blood cells and tissue culture cells as models for various biochemical reactions need to be fostered. Methods that will allow safe administration of tracer doses of the relevant agents need to be developed. Much more interdisciplinary work is required. Scientific workers dealing with drugs and other xenobiotic agents need to be trained in the principles of human biochemical variation and the underlying genetics. Human geneticists need to collaborate with clinical pharmacologists who are knowledgeable in pharmacology and pharmacokinetics. Both human geneticists and clinical pharmacologists need to have sophistication in clinical medicine and human biology to be able to design safe studies and discern potential problems in interpretation. Biochemists need to pay more attention to *human* biochemical variation of enzymes involved in drug metabolism. The large number of different talents required, with the attendant difficulties in collaboration and coordination, will make a rapid flowering of pharmacogenetics and ecogenetics difficult. Training programs, interdisciplinary workshops, and summer courses are needed to advance this field. It is hoped that the current conference will be one step in this direction.

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A. Pharmacogenetics

I. Multifactorial Pharmacogenetics in Man

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Multifactorial Inheritance and Heritability in Pharmacogenetics

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Summary. Unimodal Gaussian distribution curves are usually observed when drug metabolism is studied in human subjects. Greater similarity of identical twins as compared with fraternal twins suggests genetic influences on the metabolism of many drugs. Lack of distinct segregation into monogenically defined classes and the high heritability observed in twin data are interpreted as compatible with multifactorial inheritance influencing drug metabolism.

'Heritability' measurements as employed in quantitative genetics cannot provide data regarding the number of the genes involved nor of their mechanisms. The origin and definition of the 'heritability' concept is presented. Heritability values for drug metabolism derived from twin studies alone can be misleading since genetically identical persons may search out similar environments.

Unimodal distribution curves may be obtained with the operation of only a few allelic or nonallelic genes under certain conditions. Probably only a few genes contribute to a large portion of the genetic variation of drug metabolism. Attention to the individual components of drug metabolism by the study of drug-metabolizing enzymes and of drug metabolites therefore may detect previously unrecognized Mendelian variants. Family studies in man will be essential to detect such variation. Pharmacogenetic studies may become models for the analysis of resolving multifactorial traits into their underlying major Mendelian genes.

Monogenic traits that segregate in families produce few difficulties in genetic analysis. Thus, classic pharmacogenetic traits such as pseudocholinesterase variants and acetyltransferase polymorphism clearly exhibited multimodal variation when family investigations were done. Definite phenotypic classes reflecting the genotype were found in these traits with little overlap between categories (Motulsky, 1965). Glucose-6-phosphate dehydrogenase deficiency is an X-linked pharmacogenetic trait and deficient males can clearly be distinguished from G6PD normal males. The data are less clear among females. Three classes are

expected with X-linked inheritance: homozygous abnormal, heterozygote, and homozygous normal females. No clear segregation of these three genetic classes for G6PD deficiency was possible. However, each genetic category had a characteristic mean enzyme activity with much overlap between normals and heterozygotes with partial enzyme deficiency.

Most pharmacogenetic traits, unlike the polymorphisms for acetyltransferase, pseudocholinesterase, and G6PD deficiency, do not exhibit multimodal variation. When the results of drug metabolism or of drug response for a population are plotted, a unimodal Gaussian curve is usually obtained indicating a graded characteristic (see WHO report, 1973). The majority of the population has an 'average' response in contrast to monogenic traits where the frequency of the different phenotypes is determined by the frequency of the involved genes.

Without genetic studies, no conclusion regarding the possible genetic significance of unimodal curves can be made. Nongenetic causes of variation such as laboratory variation, nutritional variation, and different physiologic states can produce variability in drug responses. The finding that monozygous twins were more alike than dizygous twins led to the inference that multifactorial inheritance controls the disposition of most drugs. The concept of multifactorial inheritance implies the operation of an unspecified number of genes with unknown mode of action acting together with undefined environmental and random factors. The inference of multifactorial inheritance was based on the lack of multimodal variation (suggestive of single-gene inheritance) together with evidence for genetic control of the particular trait from the twin studies. Family investigations have only been rarely performed in these instances. Thus, the hypothesis of multifactorial inheritance was arrived at by exclusion and not by rigorous positive evidence.

As has been demonstrated for normal variation in activity for many enzymes with electrophoretic variants (see Motulsky, 1970, for references), a unimodal curve does not necessarily indicate multifactorial inheritance. In such instances, each electrophoretically defined enzyme type, usually reflects a single gene-controlled allelic variant. Each allelic variant has a characteristic mean enzyme activity. The wide range of unimodally distributed enzyme activity for a given enzyme in the normal population therefore may sometimes be explained by the existence of several overlapping curves, each with a mean activity characteristic for a given allelic polymorphic variant of the enzyme. As an example, if there are two allelic variants A and B of a given enzyme, there would be three genotypic classes, AA, AB, and BB. If the mean activity of the enzyme under specification of the B allele is higher than that of the A allele, three overlapping curves reflecting the activity of the AA, AB, and BB genotypes, respectively, are obtained. A seemingly unimodal curve therefore may reflect the action of only two allelic variants. Thus, high twin resemblances and a unimodal curve of variation do not necessarily mean multifactorial inheritance.

It can also be shown that additive interaction of only a few different genes can produce a single bell-shaped distribution curve. Thus, relatively few allelic genes can lead to a unimodal distribution curve. The usual assumption that a unimodal curve suggests multifactorial inheritance therefore does not necessarily hold true. In fact, an analysis of the underlying basis of a unimodal curve might be

approached by conventional Mendelian techniques used for the study of monogenic characteristics. Such approaches require the study of the underlying single-gene-determined biochemical, immunologic, or physiologic components that may contribute to the phenotype.

These concepts suggest that genetic variation in drug metabolism may be under the control of only a few identifiable biochemical reactions that in turn are governed by relatively few genes. Minor genetic variation also occurs and presumably relates to the totality of biochemical variation that makes each person unique and may be considered as the 'genetic background.' The genetics of most pharmacogenetic phenomena may be less complex than that of most other multifactorial traits, and the number of major genes contributing significant variation and potentially identifiable in the laboratory may be small. Since we know more about the biochemistry of drug metabolism than about the biochemistry of other multifactorial traits the analysis of pharmacogenetic problems may become a model for the understanding of more complex multifactorial phenomena by focusing attention on underlying biochemical mechanisms.

Every attempt should therefore be made to analyze pharmacogenetic phenomena by biologic methods that may identify monogenic phenomena rather than by statistical techniques alone. Family studies are usually required to identify the operation of Mendelian genes. Twin studies alone will not be sufficient. The phenotype selected for such studies therefore should be more likely to yield biochemical genetic resolution than a characteristic of a higher order such as drug half-life. Traits such as half-life or steady-state blood levels are remote from gene action and subsume many enzymatic and metabolic subcomponents, each with potential genetic variation. Potentially useful approaches for genetic studies with Mendelian variation involve the study of drug metabolites in urine and the direct enzyme assay of various types of blood cells.

Pharmacogenetic studies in animals are easier and may provide hints regarding gross pharmacogenetic variation. However, in human pharmacogenetics the proper study of mankind is man! Species variability and the impossibility to demonstrate intraspecies variability by studies in species other than man make human studies essential.

Problems of Heritability Measurements

The concept of 'heritability' is widely used in quantitative genetics and has been increasingly employed in studies of pharmacogenetics. It should be recalled that the concept was introduced by animal breeders who were interested in increasing production of economically useful traits such as milk yield by cows and egg laying by chickens (Falconer, 1960). The term 'heritability' was developed in a search for a reliable phenotypic value as a guide to those groups of animals that yielded the highest eonomic output in their offspring. In domestic animal breeding the environmental variables can be randomized or specifically controlled. Unfortunately, environmental variables cannot be readily controlled in many areas of human genetics. Nevertheless, in human pharmacogenetics more standardization of environments is possible than with many other human traits.

A short review of the technical terminology and its meaning may be useful (Cavalli-Sforza and Bodmer, 1971). The total variance of a quantitative trait is its squared standard deviation. The total of phenotypically observed variance is comprised of a genetically determined variance and an environmental variance. Random factors are usually omitted from the analysis. The genetic variance consists of additive variance, dominance variance, and interaction variance. The additive variance relates to the action of an unspecified number of genes that add equal increments to the trait. Dominance variance is the variance contributed by heterozygotes Aa if the quantitative effect of Aa is not exactly intermediate between the homozygotes aa and AA. The interaction variance refers to interaction between the genotype and the environment in such a manner that different genotypes are affected differently by the same environment.

Heritability (h_2) is a technical term and refers to the fraction of the total phenotypic variance that can be ascribed to the genetic variance

$$\left(h_2 = \frac{\text{genetic variance}}{\text{total phenotypic variance}}\right)$$

Heritability values can range between 0 and 100%. Often, quantitative geneticists differentiate between broad and narrow heritability. Broad heritability refers to the effect of the total genetic variance while narrow heritability only considers the additive genetic variance as a fraction of the total phenotypic variance, so the broad heritability will usually be higher than the narrow heritability.

There are a series of general problems with heritability measurements in human genetics. Heritability is a population statistic and is meaningless if applied to a single individual. Heritability is a ratio $\left(\frac{\text{genetic variance}}{\text{total phenotypic variance}}\right)$ and fluctuates in different environments. It will be higher if there is restricted environmental variation, but will be lower with restricted genetic variation. Various studies have shown that heritability fluctuates with time, and varies in different populations and with different test systems. Different ways of calculating heritability may give different values. Heritability provides no information about genetic differences *between* groups. A high heritability for a trait in a population does not imply that different average values for that trait in another population must be caused by genetic differences. High heritability is fully compatible with a change in a trait resulting from environmental manipulation. For instance, stature has a high heritability and yet stature has increased in Western countries over the past 2—3 generations with better nutrition.

When heritability measurements are calculated from twin studies alone, additional problems need to be considered. The analysis of twin heritability studies implies that identical environmental influences act on both identical and fraternal twins. Since monozygous twins, because of their genetic identity, may develop similar living habits that may influence drug metabolism even if living in different households, a twin study comparing identical and nonidentical twins does not absolutely prove the operation of genetic factors on drug metabolism. Twin studies also do not allow the separation of the effects of dominance variance from additive genetic variation.

Considering these limitations, one should be careful in the use of heritability values (Feldman and Lewontin, 1975). A high heritability derived from twin

studies strongly suggests that genetic factors may be operative on a given trait. Further genetic studies, however, are very desirable. The time is ripe for family studies on the components and mechanisms involved in drug absorption, metabolism, membrane and organelle interaction, detoxication, and excretion. The existence of extensive genetic polymorphisms in the human population suggests a rich yield of data that explain genetic variability in response to drugs when various enzymes and proteins are studied directly.

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