ASSESSMENT AND

GENETIC SUSCEPTIBILITY TO TOXIC CHEMICALS

by
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The range of human diseases and abnormalities contains, at one extreme, conditions regarded as inherited, e.g. sickle cell anaemia, phenylketonuria and haemophilia, and, at the other extreme, typically environmental diseases, such as anthrax and typhus. However, along this continuum are many disorders in which both genetical and environmental factors are apparently important. The genetic factors determine an individual's susceptibility to disease. The environmental agents are responsible for the manifesting of symptoms.

The elements of the situation were explained by Harris (1) in the form of two circles, a larger one enclosing a much smaller circle. The outer circle represents a population while the inner circle represents a subgroup of individuals genetically predisposed to develop a particular kind of disease. The two lines drawn from the centre to the periphery in a wedge shape divide the entire population into those individuals who happen to be exposed to environmental factors that tend to elicit the abnormality and those who are not exposed. Only the small segment of the population who are both genetically predisposed and subject to the unfavourable environmental situation actually develop the clinical disorder.

Pharmacogenetics and ecogenetics are scientific disciplines which are both devoted to the problem of the genetical predisposition to drugs and environmental agents. Pharmacogenetics was founded in the mid-1950s, when the differentiation of effects provoked by therapeutic doses of suxamethonium in a group of human beings was found to be genetically determined. In addition, the different variants of glucose-6-phosphate dehydrogenase (G-6-PD) were found responsible for the differentiation in susceptibility of red blood cells to haemolytic effects of some drugs. Evidence indicated that people who are carriers of some G-6-PD variants can manifest the haemolysis even after ingestion of substances which are normally fully harmless, e.g. fava beans.

Some years later, a wide variability was found in the extent to which individuals inactivate isoniazid and some

other drugs by acetylation. At first, the acetylation polymorphism was regarded harmless. In the opinion of several researchers, the same handicaps were bound to both the status of rapid and slow inactivators of isoniazid. After analysing this situation, Vogel, as cited by Motulsky (2), proposed the term "pharmacogenetics" which was generally accepted by researchers. Recently, great efforts have been made to discover additional monogenic traits bound to unusual effects of drugs but have met with little success. Vogel's ideas were revived when Vessel & Page (3) demonstrated that the rate of metabolism of most drugs appears genetically influenced by polygenic control. This finding is apparent from a number of studies on identical and fraternal twins. The majority of normal inter-individual differences show continuous variation. Continuous variation cannot be explained on the basis of single genes; if hereditary, it must depend on the combined action of a number of genes. Part of continuous variation is due to inheritance, part to environmental factors.

Today, pharmacogenetics is no longer a field in which extraordinary reactions to drugs are discussed but is a discipline of central significance for pharmacology and toxicology. The introduction of genetic concepts to experimental pharmacokinetics forecasts that very interesting data will be obtained in the future.

Recently, pharmacogenetic ideas were transferred to environmental problems. In 1971, the term "ecogenetics" was first used by Brewer, as cited by Motulsky (2). Ecogenetics is concerned not only with toxic agents but also teratogenic, mutagenic and carcinogenic substances. However, the list of research works analysing genetically influenced variation in response to environmental agents is still very modest. Ecogenetic problems will probably inspire the specialists involved in resolving questions of occupational health and industrial toxicology.

This paper will present some genetic problems which seem to play a role in human exposure to toxic chemicals. First, several pharmacogenetic problems related to major inherited traits and polygenic variation will be discussed. Second, selected ecogenetic problems will be reviewed. Third, some bio-ethical problems in pharmacogenetics and ecogenetics will be analysed.

Pharmacogenetic Problems

Serum cholinesterase variants and succinylcholine sensitivity

In most individuals, the neuromuscular blocker suxamethonium has a duration-of-action rate of only a few minutes because the drug is rapidly metabolized by plasma cholinesterase. In rare individuals, suxamethonium induces a neuromuscular block of two to three hours duration because the drug is not metabolized in the customary way. In such individuals, the enzyme exists in a variant form which cannot hydrolyze this substrate. The variants unable to hydrolyze suxamethonium were first described by Lehman & Ryan (4), Kalow & Genest (5) and Goedde et al. (6). Evidence quickly established that only two thirds of individuals who show susceptibility to suxamethonium are carriers of an atypical, dibucaine-resistant allele. People who show prolonged apnoe following suxamethonium can also be carriers of an allele which is responsible for the fluoride-resistant cholinesterase variant or of a silent gene responsible for very low enzyme activity. In addition, about one-third of individuals who exhibit pathological reaction to suxamethonium seem to have a normal enzyme. In a case of abnormal susceptibility to suxamethonium, Thompson & Whittaker (7) were able to show a decreased tolerance of cholinesterase to chloride (chloride sensitivity). Further progress was recently made when Goedde & Agarwal (8) used succinyldicholine as a cholinesterase substrate. If the allele responsible for the synthesis of cholinesterase, which slowly metabolizes suxamethonium, is symbolized as E_1Su, all the other alleles and their combinations discovered to date can be summarized as presented in Table 1.

Previously, the fact that cholinesterase is also synthesized in a second locus (E_2) was not considered. This synthesis increases the cholinesterase activity in plasma in some individuals (E_2 genotype) but is unimportant in the susceptibility of the enzyme to suxamethonium action. Table 2 presents all alleles responsible for cholinesterase synthesis and their frequencies in the population.

Table 1. Possible combinations of alleles responsible for the synthesis of plasma cholinesterase (8)

$E_1^U E_1^U$	$E_1^f E_1^S$	$E_1^U E_1^A$
$E_1^A E_1^A$	$E_1^f E_1^{Su}$	$E_1^U E_1^f$
$E_1^f E_1^f$	$E_1^A E_1^S$	$E_1^U E_1^S$
$E_1^S E_1^S$	$E_1^A E_1^{Su}$	$E_1^U E_1^{Su}$
$E_1^{Su} E_1^{Su}$	$E_1^S E_1^{Su}$	$E_1^f E_1^A$

where: E_1^U is usual, E_1^f is fluoride-resistant; E_1^A is atypical, dibucaine-resistent; E_1^S is none (silent gene), and E_1^{Su} is slowly metabolizing suxamethonium

Table 2. Variants of plasma cholinesterase (pseudocholinesterase) (9)

Locus	Gene	Enzyme variant	Frequency of homozygotes
First	E_1^U	Usual	94%
	E_1^A	Atypical, dibucaine-resistant	1 in 2500
	E_1^f	Fluoride-resistant	Very rare
	E_1^S	None (silent gene)	Very rare
	E_1^{Su}	Slowly metabolizing suxamethonium	Unknown
Second	E_2^-	C_5 electrophoretic bend absent	90%
	E_2^+	C_5 electrophoretic bend present	10%
Unknown	E^{Cl}	Incompletely resolved chloride sensitivity	Unknown
	E_{Cyn}	High activity	Very rare

The role of cholinesterase polymorphism in the susceptibility to pesticides, which are cholinesterase inhibitors, is still uncertain. The influence that different alleles exert on enzyme plasma concentration may influence the resistance of their carriers to inhibition.

Glucose-6-phosphate dehydrogenase deficiencies and primaquine sensitivity

At least 80 distinct variant forms of glucose-6-phosphate dehydrogenase (G-6-PD) have been identified (10). All these variants do not cause haemolysis with primaquine and other drugs. Kirkman (11) has pointed out that, in

general, those variants with activity less than 30% of normal value are the ones regularly associated with haemolytic reactions to drugs. The mechanism which accounts for red cell haemolysis is not known, but the reduced level of G-6-PD activity clearly makes these cells less efficient in generating NADPH, the reduced co-factor for glutathione reductase. An adequate supply of glutathione seems to be critical in maintaining the integrity of the erythrocyte membrane. Assays of red cell G-6-PD levels in Negro populations reveal a striking difference (1). In healthy males, two clearly distinct classes of individuals can be recognized: those with normal levels of the enzyme and those who are deficient. Within each group, considerable variation is found, but the two distributions hardly overlap. A quite different situation is found in females. Here, a more or less continuous variation in levels of the different individuals is observed. All values may be found from the normal level characteristic of the non-deficient males to the low level characteristic of the deficient males.

Males are more likely to show enzymatic deficiency and drug sensitivity because the gene determining the characteristics of G-6-PD is carried on the X chromosome. The X chromosome of the males can be normal or defective, and the two male genotypes "reactor" or "normal" are expected. Females may be classified into three groups, "normal", "intermediate" or "reactor", depending on the presence of two normal X chromosomes, one normal and one defective, and two defective X chromosomes, respectively. Most of the heterozygous females show, however, a measurable deficiency of the enzyme, but a few heterozygous females also show quite a marked deficiency, as do hemizygous men. An explanation for the variable expression in heterozygous women is found in the phenomenon of random X chromosome inactivation, the basis of the Lyon hypothesis. Other drugs besides primaquine may induce haemolytic crisis in G-6-PD-deficient subjects (Table 3).

Many variants of G-6-PD occur. They differ from one another in their qualitative characteristics (e.g. electrophoretic mobility, K_Mm thermostability). Many of these variants are associated with some degree of enzyme deficiency. At least one variant (Gd Hektoen) is associated with a marked elevation in activity. In addition, certain variants are associated with a chronic

haemolytic anaemia (so-called non-spherocytic haemolytic disease), which is present even in the absence of any obvious precipitating factors such as a particular drug.

Table 3. Drugs and other agents that can cause clinically significant haemolysis in G-6-PD-deficient individuals (11)

Acetanilide	Pentaquine
Diaminodiphenylsulfone	Phenylhydrazine
Fava beans	Promaquine
Furaltodone	Quinidine
Furazolidone	Quinocide
Naphthalene	Sulfacetamide
Neosalvarsan	Sulfapyridine
Nitrofurantoin	Sulfmethoxypyridazine
Nitrofurazone	Salicylazosulfapyridine
Nitrofurazone	Thiazolsulfone
Pamaquine	Trinitrotoluene

Slow and rapid acetylation of isoniazid

The following examples illustrate how the same genetic polymorphism of the enzyme N-acetyltransferase influences the metabolism of a wide variety of drugs. Acetylation is a controlling factor in the rate of metabolism of several drugs, the acetylated drug being more easily excreted by the kidney than is the free drug. In Western populations, about half the individuals are rapid acetylators and half are slow acetylators. Individuals can be reliably classified by comparing serum levels after a standard dose. After a single dose of isoniazid, plasma concentrations have a bimodal distribution that contrasts markedly with the unimodal distribution of concentrations after the administration of salicylate, which is not influenced by a metabolic polymorphism. One consequence of the isoniazid metabolism polymorphism is that neuropathy as a complication of isoniazid therapy is more likely to occur in slow acetylators. Recent evidence shows another side to the acetylator, for "rapid"

acetylators are more likely to develop hepatitis as a reaction to isoniazid. A coincidence of acetylation has been demonstrated between isoniazid and sulfadimidine *in vivo* and also with hydrallazine *in vitro*. Among hypertensive subjects, slow acetylators have been found to require smaller doses of hydrallazine for blood pressure control, and they more commonly show toxic signs and develop antinuclear antibodies.

Human liver N-acetyltransferase catalyzes the acetylation of a number of drugs (Table 4).

Table 4. Some drugs inactivated by acetylation in humans (12)

Group I	Acetylated by INH N-acetyltransferase
	Isoniazid (INH)
	Sulfamethazine
	Hydrallazine (Apresoline)
	Diaminodiphenylsulfone (Dapsone)
	Phenelzine (Nardil)
Group II	Acetylated, but importance of INH N-acetyltransferase polymorphism uncertain
	Sulfamethoxypyridazine
	Sulfisoxazole (Gantrisin)
	Sulfadiazine
Group III	Acetylated mainly by systems other than the INH N-acetyltransferase system
	p-aminosalicylic acid
	p-aminobenzoic acid
	Sulfanilamide

Other hereditary disorders with altered drug effects

Another inherited condition revealed only by the administration of an anaesthetic is malignant hyperthermia. This disorder is characterized by a rapid rise in body temperature and progressive muscular rigidity

following anaesthetics (halothan, methoxyflurane or ether). The basic defect is unknown but is probably related to intracellular calcium metabolism. The condition is inherited in an autosomal dominant fashion and is quite rare, about 1:20 000.

Anaesthesia is a potential hazard for individuals with sickle cell haemoglobin; hypoxia may cause intravascular sickling followed by infarction.

Inherited variation in the structure of haemoglobin may predispose a patient to undesirable drug effects, such as methaemoglobinaemia and haemolysis.

The occurrence of marked methaemoglobinaemia following drug administration suggests that some individuals cannot reduce this substance at a normal rate. A single case precipitated by phenacetin has, however, been shown to be associated with partial failure of de-ethylation of the drug to form paracetamol with consequence accumulation of other metabolites. The finding that a sister of the propositus had the same defect indicates an inherited cause by presence or absence of a major inherited trait (13). The chief objection is that phenacetin de-ethylation is catalyzed by ordinary microsomal mixed-function oxidases which are probably under polygenic control.

Polygenic variation

Three main types of genetic variation exist. The first is variation due to major genes. The second is polymorphic, that is, alternative common genes can occur at the same locus in the population. The third is multifactorial or polygenic inheritance, the genetic variation due to combined action of a number of genes.

Several relatively common human disorders conform to a multifactorial mode of inheritance showing continuous variation. Part of the continuous variation is due to inheritance, part to environmental factors. In some instances, the environmental influence is greater than the inheritable component.

The rate of metabolism of most drugs appears to be genetically influenced by polygenic control, as apparent from a number of studies on identical and fraternal

twins. Thus, identical, but not fraternal, twins show very close agreement in their handling of phenylbutazone, antipyrine, ethanol, halothan and nortryptyline. The implication of the twin and family studies is that the quantity of the rate-limiting enzyme in the liver microsomes is genetically influenced. Support also appears for the polygenic nature of the influence over nortryptyline metabolism. As shown by Asberg et al. (14), relatives of propositi who develop high steady-state plasma levels themselves develop steady-state levels that are high but normally distributed. The existence of polymorphism is thereby excluded because such would have produced a bimodal or trimodal distribution.

Ecogenetic Problems

Variation in aryl-hydrocarbon hydroxylase inducibility

A component of the mixed-function microsomal oxidase system, designated arylhydrocarbon hydroxylase (AHH), has recently attracted the attention of many investigators. This enzyme system metabolizes, and is induced by, a variety of compounds, including the polycyclic aromatic hydrocarbons. Some of these compounds (e.g. benz(a)pyrene) are converted to metabolites which have carcinogenic potential while others are converted to metabolites which are noncarcinogenic.

In experimental animals, the AHH enzyme system can be induced using several different chemical substrates. The level of AHH inducibility has been shown to be genetically determined in certain strains of mice, and genetic inheritance shown to follow a dominant mode of transmission when 3-methylcholantrene is used as an inducer. Kellerman et al. (15) have suggested that the level of enzyme inducibility in human lymphocytes is genetically regulated. Guirgis et al. (16) compared levels of AHH activity in lymphocytes of lung cancer patients with normal controls. Lung cancer patients exhibited elevated levels of both AHH activity per se and AHH activity for total cellular DNA than did controls. Lung cancer may be much more likely in smokers who are genetically endowed with high inducibility of the activating enzyme AHH. Although serious doubt has recently been cast on this hypothesis, the possibility remains that genetic polymorphism of this type exists (17).

Hypersusceptibility to carbon disulfide (CS_2)

Indications show that certain people exhibit genetic hypersusceptibility to CS_2. During exposure to CS_2, hypersusceptive men eliminate CS_2 metabolites more slowly than do other exposed people. A test has been developed to detect hypersusceptibility in persons during the job preplacement medical examination. Djuric et al. (18) proposed a perspective study of individuals before and after exposure to CS_2 and under situations where environmental conditions are accurately known.

Hypersensitivity to organic isocyanates

The rapidly expanding application of aliphatic and aromatic isocyanates has resulted in widespread exposure of increasing numbers of workers to these compounds. A fairly common response from the inhalation of these mists and vapours, following acute toxic symptoms, is an asthma-like syndrome that develops on subsequent exposure to even minute amounts of isocyanate. This "immediate" type of hypersensitivity resembles the "wet" allergic reactions commonly associated with "hay fever" or plant pollen reactions. Tests were developed to detect those individuals who are hypersensitive to these chemicals prior to exposure because the control of isocyanate exposure below permissible industrial air limits is not possible. The hereditary basis of susceptibility is unknown (19).

Bioethical Problems in Pharmacogenetics and Ecogenetics

Job preplacement examination

During the job preplacement medical examination, testing has been suggested to detect workers hypersusceptible to industrial chemicals. This testing may aid job assignment by considering the innate, individual characteristics of the worker, thus reducing unnecessary health risk (19).

The practical application of this suggestion may include some risk. A situation may develop in which a harmless deficit of an enzyme or a protein could be used as argument to discriminate against racial or ethnic groups with high frequencies of such deficits. According to

Motulsky (20), a recommendation was issued against introducing into the United States Army individuals exhibiting sickle cell trait or G-6-PD deficiency in red blood cells. In this case, entry into the United States Army would have been more difficult for Negroes who commonly have both traits.

Investigation of new drugs

Species differences in response to drugs must be considered in the extrapolation of pharmacological and toxicological data obtained from experimental animals to humans. In recent years, studies of drugs in monkeys have been commonly considered valid predictors of metabolic disposition in humans. However, this view has been questioned; in many instances, metabolic data obtained in the monkey have been of no better predictive value in man than those of the dog. Genetic factors playing an important role in explaining individual differences in drug metabolism deserve much more observation in human beings than is actually used. Marked individual variation exists in the metabolism of drugs which are handled primarily by enzymes in liver microsomes. Some patients metabolize a drug so rapidly that therapeutically effective blood and tissue levels are not achieved while others metabolize the same drug so slowly that toxic effects result.

Individual regimens for drugs

Detailed information about the physiological disposition of drugs in humans must be readily available to help clinicians prescribe dosage schedules which will provide optimal efficiency and minimal risk of toxicity. The time is approaching when the calculation of dosage for certain drugs is necessary for each individual patient.

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