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FROM PHARMACOGENETICS AND ECOGENETICS TO PHARMACOGENOMICS

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SUMMARY

The origin and development of pharmacogenetics are traced with emphasis on early hints by Garrod, Haldane, and later by RJ Williams. The field was delineated by Motulsky in 1957 and described as pharmacogenetics by Vogel in 1959. Kalow's monograph (1962) definitely established the discipline. Resemblance of identical twins in drug metabolism as compared with non identical twins (Vesell, 1970's) established the general importance of polygenic inheritance in disposal of many drugs. Ecogenetics was defined by Brewer in 1971 as dealing with genetic variation affecting the response to any environmental agents with emphasis on xenobiotics. More recent developments have broadened pharmacogenetic approaches to include novel genomic techniques with introduction of the term pharmacogenomics in the 1990's. Genetic and genomic approaches (toxicogenetics and toxicogenomics) are also being applied in the "environmental genome project". The interaction of genetic variation with dietary factors led to the field of Nutritional ecogenetics (Nutrigenomics) which relates the role of genetics to nutritional requirements and nutrition-mediated susceptibility to chronic disease. The total promise of pharmacogenomics is often overstated. The field is likely to have an impact on choice of drug therapy and avoidance of adverse events but is unlikely to lead to a revolution in therapeutics. Aspects of pharmacogenomic approaches and its applications including problems of premature commercialization are discussed.

A. *Garrod (1857-1936). Founder of human biochemical genetics*

The emergence of Pharmacogenetics in the 1950's was solidly based on scientific developments in human biochemical genetics.

Key words: History of Pharmacogenetics – Pharmacogenomics – Ecogenetics – Nutrigenomics – Environmental Genome Project – Current Status of pharmacogenetics

Archibald Garrod at the turn of the 20th century described several familial inborn errors of metabolism such as albinism and alkaptonuria which he ascribed to alternative pathways of metabolism[1]. He consulted Bateson – an early geneticist (the man who coined the term genetics) who pointed out that the familial nature of these conditions together with frequent consanguinity of unaffected parents was consistent with the mechanism of Mendelian recessive inheritance. The term "gene" to denote Mendelian factors was first used by Johannsen in 1909[2] but Garrod tended to avoid the word in his later writings. While he knew about enzymes and clearly set the stage for the one gene – one enzyme hypothesis of the 1940's[3], he did not relate the Mendelian factors responsible for the inborn errors of metabolism to altered enzyme function. Nor did he suggest that the normal condition of such a factor (ie. a gene) specified the nature of an enzyme. Nevertheless, Garrod clearly foresaw the role of genetic factors in most diseases and in human variation far ahead of his time. He enunciated the concept of chemical individuality of humans and other animals with special reference to intermediate metabolism and its chemical basis. Garrod stated, "even those idiosyncrasies with regard to drugs and articles of food which are summed up in the proverbial saying that what is one man's meat is another man's poison presumably have a chemical basis." Beyond the rare chemical "malformations" that he held responsible for inborn errors of metabolism he suggested that minor metabolic differences were leading to variable amounts of the final or intermediate products of metabolism. Because such slight metabolic deviations were difficult to detect they would usually escape attention.

Near the end of his career in 1931, Garrod – by now a major figure in British medicine - who held a prestigious chair at Oxford University - published a lengthy essay as a book, *The Inborn Factors in Disease*, where he generalized his concepts of inborn chemical individuality to include most diseases[4]. As in his earlier work, he again pointed to potential adverse reactions in only some subjects and stated . . .

> *substances contained in particular foods, certain drugs, and exhalations of animals or plants produce in some people affects wholly out of proportion to any which they bring about in average individuals. Some effects vary from a slight and temporary discomfort or morbid syndromes which amount to severe or fatal illness."*

As had happened with his earlier work on inborn errors of metabolism, Garrod's ideas despite his prominence attracted little attention. In 1989, the Oxford University Press (OUP) reissued the 1931 book with extensive commentary by medical geneticists Charles Scriver and Barton Childs[5]. In 1963, Garrod's earlier work on inborn factors of metabolism (1908) had been reprinted by OUP with a long supplement by Harry Harris - a biochemical geneticist[6].

J.B.S. Haldane (1892-1964) – 20th century biochemical geneticist

The well known British geneticist JBS Haldane in 1954 summarized the status of the still young field of biochemical genetics in a book, *The Biochemistry of Genetics*[7] which presented a review of relevant findings in micro-organisms, plants, animals and humans. In summing up he predicted "that the future of biochemical genetics applied to medicine is largely in the study of diatheses and idiosyncrasies, differences of innate make up which do not necessarily lead to disease but may do so."

Initial pharmacogenetic examples

Thus, the two British founders of biochemical genetics, Garrod and Haldane, set the stage for the delineation of the field of pharmacogenetics. Soon after Haldane's 1954 book was published, a University of Chicago group demonstrated that the occasional hemolytic anemia due to the anti-malarial drug Primaquine (observed in US Soldiers of African origin) was caused by a red cell defect affecting glutathione metabolism[8]. Subsequently, this abnormality was demonstrated by Barton Childs to be X chromosome-linked[9] and shown by Carson to be caused by deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6pd)[10]. About the same time, Lehman and Ryan in the UK demonstrated that prolonged apnea following succinylcholine administration to relax muscles during surgery could be explained by low levels of the liver enzyme pseudocholinesterase (butyrylesterase) that was re-

quired to metabolize the drug[11]. Family studies suggested autosomal recessive inheritance. Kalow and Staron[12] showed this familial defect to be caused by an inherited qualitative enzyme abnormality which could be differentiated from the normal enzyme by certain inhibitors and allowed clear definition of heterozygotes and homozygotes from those not carrying the variant enzyme. After Lehman presented his data at the First International Congress of Human Genetics in Copenhagen in August 1956, I discussed these findings at that meeting as conceptually analogous to the genetically determined hemolytic anemia induced by primaquine and suggested the importance of such findings as models for the genetic basis of other adverse drug reactions.

Definition of pharmacogenetics

With my background in hematology, I was serving in the mid 1950's on a subcommittee on blood dyscrasias of the Committee on Research of The American Medical Association. Invited by this committee, I wrote an article for the Journal of The American Medical Association with the programmatic title: "Drug Reactions, Enzymes and Biochemical Genetics"[13]. In this 1957 paper, the confluence of genetics, biochemistry and pharmacology was set out to define the genetic principles of this new approach to explain some unexpected and unexplained responses to drugs. More generally, the importance of monogenic drug reactions as a model for the interaction of heredity and environment in the pathogenesis of disease was emphasized. A genetic trait alone such as G6pd deficiency that predisposes to an adverse drug reaction does not impair health nor does a standard dose of the offending drug in those with normal G6pd activity. Only those with G6pd deficiency become ill ie. developed hemolytic anemia under such circumstances. The drug is the specific environmental agent that caused disease (ie. the drug reaction) only in a genetically susceptible person. The term "Pharmacogenetics" for this field of study was not yet used and was later introduced by Friedrich Vogel in a review paper on human genetics in 1959[14].

At the First International Human Genetics Congress, in Copenhagen an American biochemist RJ Williams (1893-1988) presented an invited paper: "Biochemical Genetics and its Human Implications"[15]. Williams was a nutritional biochemist, the discoverer of pantothenic acid, a member of the National Academy of Science, and President of the American Chemical Society in 1957. He published a book, *Biochemical Individuality* (1956)[16] which provided additional documentation. Gathering data from anatomy, enzymology, endocrinology, pharmacology, and particularly from nutrition, he documented frequent biological and biochemical differences between normal individuals stressing that every person was unique and a "deviate" in some sense. Williams' interest in pharmacologic data was inspired by his own idiosyncratic reaction to morphine manifesting as "excruciating mental frenzy"[17]. While Williams' quoted Garrod, as well as Beadle and Tatum, he was admittedly not knowledgeable about genetics even though he strongly emphasized the role of heredity. Working in a Biochemical Institute at the University of Texas in Austin where no medical school existed, Williams' impact on further research in human genetics, biochemistry, nutrition, and medicine remained minimal, largely because he did not couple his general ideas about heredity with the emerging technology of biochemical genetics. He failed to attract pharmacologists, nutritionists and others to investigate these ideas. The genetic paradigm did not yet fit the research programs in these fields. His 1956 book was reissued in 1998 with a Foreword, Afterword and a Biographical memoir[18].

Werner Kalow's monograph on Pharmacogenetics

The field of pharmacogenetics became definitively established as a unique discipline with Werner Kalow's publication of his monograph *Pharmacogenetics* (1962)[19] that reviewed all aspects of the field. My 1957[13] and Vogel's 1959 review[14] were cited as was Williams' book on biochemical individuality. Kalow was a pharmacologist who continued to devote his career to pharmacogenetics and played a leading role in introducing this field into the biomedical sciences. He remains a major figure who made many conceptual and technical contributions[20].

In earlier years, considerable work on what could be categorized as pharmacogenetics had already been done in several mammalian species. Special attention had been given to various

inbred mouse strains with different genetic backgrounds and was mentioned in Kalow's book. In 1963, Hans Meier, a scientist from the well known Bar Harbor mouse laboratory summarized such work in a book, *Experimental Pharmacogenetics*[21], where he stressed the potential role of animal investigations that could not be done on humans but might help in solving problems in human pharmacogenetics. Even though mouse genetics had been useful for better understanding of human genetics in general, few problems of human pharmacogenetics have yet directly benefited from mouse or other animal studies. Despite many similarities between the human and mouse genome, species differences in pathways mediating xenobiotic metabolism remain.

Discovery of more monogenic pharmacogenetic traits

Progress in identifying adverse drug reactions caused by single gene mutation (monogenic inheritance) was slow in the years following my publication of JAMA article in 1957[22]. Novel findings of monogenic pharmacogenetic traits in the 1960's included slow inactivation of isoniazid that predisposed to peripheral neuritis by a common acetyltransferase variant[23] drug induced hemolytic anemia caused by unstable hemoglobins[24], malignant hyperthermia following inhalation anesthesia[25] and resistance to coumadin anticoagulants[26]. The paucity of detecting other monogenic examples could be explained by the rarity of many of such traits as well as lack of genetic interest among pharmacologists and physician - scientists. Moreover, family studies often required administration of a drug to healthy family members and were therefore difficult logistically. Thus, few relevant studies were performed.

Twin studies in pharmacogenetics

Vesell and Page in the late 1960's (and later others) utilized a classic approach of human genetics and carried out twin studies with a variety of drugs[27]. The rationale was that if heredity was important in drug metabolism, genetically alike identical twins would resemble each other more closely in measures of drug metabolism as compared with non identical twins who only shared one half of their genes. The results from such twin stud-

ies confirmed the genetic hypothesis since for most drugs investigated there was much more similarity between identical twins when quantitative parameters of drug metabolism were measured. Even though multiple environmental factors are well known to affect drug disposal, the twin studies pointed to the importance of genetic variation in drug metabolism. These twin data and the variability of drug metabolism (such as differing blood levels) when a standard dose of a drug was given to members of a normal population together with family studies that showed non Mendelian familial aggregation were consistent with multi-factorial inheritance involving multiple genes (polygenes) as well as environmental factors. The concept that genetic factors played a role in the metabolism of many drugs transformed pharmacogenetics from a field dealing with a few unusual adverse drug reactions to a discipline of more central importance for pharmacology and therapeutics and of potentially great interest for medicine. However, it took about 30 years to demonstrate the validity of this concept and its acceptance by the scientific community. Even today, the detailed genetic and biochemical elucidation of the many genes of small effect affecting polygenic drug metabolism requires much further study.

Since there are many potential steps in the path of a drug through the organism including absorption, plasma binding, metabolism, receptor action, and excretion, there are many sites at which genetic variability may exist. The bell shaped unimodal distribution curve obtained when the results of drug half lives (or of related measures of drug metabolism) after standard dosage are plotted for members of a population will usually relate to the action of more than one genetic variant, usually in concert with various environmental factors. These findings are in contrast to the multimodal distribution curves of monogenic traits where clear-cut differences between normals and abnormals are often observed in pharmacogenetically relevant population studies. I summarized the status of pharmacogenetics in 1971, at the 4th International Congress of Human Genetics[28].

Ecogenetics

J.B.S. Haldane – the polymath biologist with deep insight into biologic variation suggested in 1938 that "potter's bronchitis"

might be caused by a "constitutional" difference among affected potters[29]. Since the majority of potters do not die of bronchitis, an inborn susceptibility would only make some individuals liable to develop the disease. This was the first suggestion that genetic differences might predispose some individuals to an occupational disease caused by environmental injury.

In 1967, I was asked to give a lecture at a Congress of the American Medical Association (AMA) on environmental health problems that dealt with genetics and environmental health[30]. A variety of issues such as the potential role of chemical agents and radiation in mutagenesis were raised. Natural selection of human genetic traits by environmental agents such as infectious organisms (eg. hemoglobinopathies and G6pd deficiency protecting against malaria) were also discussed. A striking example of genetic-environmental interaction from our own experimental work with the red cell trait of hereditary spherocytosis in deermice – a model for the analogous condition in humans was cited[31]. When ambient temperature of the mice was raised to 35°C, these animals developed severe hemolysis with a 50% mortality after 2 weeks exposure - a demonstration of how a relatively benign trait became lethal at elevated environmental temperatures that did not harm control mice in these studies.

Based on the precedent of pharmacogenetics, Brewer in 1971 coined the term Ecogenetics to extend the concept of genetic variation to xenobiotic and environmental agents other than drugs[32]. Pharmacogenetics therefore should be considered as a sub-field of Ecogenetics and refers to genetic variation in response to drugs used for medicinal treatment. Geneticists for many years pointed out the role of genetic variation in response to any kind of environmental exposure. Drugs are only a small fraction of environmental chemicals (xenobiotics) to which humans are exposed. Other potentially toxic agents exist in the environment and may damage a fraction of the population who are genetically predisposed. The terms, "toxicogenetics" and "toxicogenomics" have been applied more recently to the role of genetic variation in response to any kind of toxic exposure. Ecogenetics and toxicogenetics have become new approaches to epidemiology in explaining why only some members of the population exposed to equal

"doses" of a damaging agent get sick. Ecogenetics now is considered an important part of public health genetics[33].

Genetically determined adverse reactions in ecogenetics may be infrequent and may be caused by a rare gene. Other variant responses may be due to genetic polymorphisms occurring in a larger proportion of the population (2% - 50%). Some ecogenetic responses may involve the action of several genes causing affected individuals to fall towards one end of a bell shaped distribution curve that relates to a quantitative response.

An International Titisee conference on Pharmacogenetics and Ecogenetics was held in 1977[34]. Topics of ecogenetic interest included problems of nutrition such as the common lactase polymorphism predisposing to lactose malabsorption, genetic hyperlipidemias, and hemochromatosis which set the stage for the field on Nutritional ecogenetics. Difficulties of assessing mutagenesis and carcinogenesis because of genetic variation affecting the metabolism of mutagenic and carcinogenic agents were discussed as well as some social and bioethical problems raised by human genetic variability. In the 1978 the status of the field was summarized by Omenn and Motulsky[35]. A wide ranging monograph on ecogenetics by E. J. Calabrese, published in 1984, covered many different genetic traits[36]. The report of a 1989 World Health Organization (WHO) consultation on Ecogenetics (with the subtitle of Genetic predisposition to the Effects of Chemicals) appeared in 1991[37].

Just as pharmacogenetics did not "catch on" until the late 1990's, ecogenetics remained somewhat neglected. The field was very broad and definitive findings that could be readily used in diagnosis and prevention were slow to emerge. The genetic point of view became more popular later when molecular and DNA methodology could be more widely applied.

Pharmacogenomics

While the concept of pharmacogenetics is over 40 years old, the term "pharmacogenomics" became popular in the middle 1990's. This development followed the introduction of genomic and molecular biologic approaches to various genetic diseases. The term "genome" refers to the totality of the genetic material

and the journal Genomics was first published in 1984. Soon it became evident that understanding of gene function ultimately required not only a physical map of the genome but needed information of the sequence of the 3 billion building blocks of DNA – a giant project. Developments in human and medical genetics led to the emerging realization that human diseases had either a definite genetic basis or often were caused by genetic susceptibilities interacting with the environment. The need for a major undertaking – the human genome project - to help in understanding and ultimately in management of disease could now be cited as justification for public investment in a big science project of this sort. Furthermore, it became clear that the availability of the full DNA sequence was going to be helpful for much of biologic and medical research. In fact, an analogy to the need for a table of the chemical elements for research in physics and chemistry can be made. The human genome project was officially initiated in the early 1990's. The project was initially carried out in the USA with public support and in the UK by the Wellcome Trust. DNA sequences obtained in the project were frequently released on the internet and made available to all scientists. Later, genome sequencing was also taken up by the private company Celera using whole genome shotgun sequencing methods in contrast to chromosome by chromosome sequencing in the public project. In the shotgun technique the entire set of chromosomes is broken into small pieces and the giant jugsaw puzzle ie. the sequence is solved using mathematical algorithms. Despite scientific disputes between the two groups, the almost complete sequence of human DNA (ie. the first draft) was separately published in early 2001[38] and should be complete in 2003.

Pharmacogenomics is the merger of pharmacogenetics with genomics and the term is often used to emphasize the novel genomic aspects of pharmacogenetics, particularly the ability to assess the action and expression of many genes at one time. Often the terms are used interchangeably. Since genomic techniques are frequently used to detect fundamental molecular abnormalities of disease, the elucidation of such defects can now guide a search for uniquely constructed drugs that specifically act upon a demonstrable molecular error. The search for molec-

ular mechanisms of disease with the aim of finding drugs to counteract disease by rational rather than empirical methods has now become one of the aims of modern pharmacogenomics.

The human genome project is based on the standard or canonical DNA constitution of a single or a few individuals. The extent of human variation which affects only a fraction of the population therefore can only be studied when the DNA of a large number of individuals is examined – a laborious and difficult undertaking. Search for polymorphism or variants that are observed in only a few percent of the population and predispose to adverse drug reactions or drug non-responsiveness therefore requires a variety of genetic and genomic techniques.

Environmental Genome Project[39]

The realization that genetic variability between individuals may influence their response to potential harmful environmental exposure led to the establishment of the "Environmental Genome Project" (EGP) by the National Institute of Environmental Health Sciences of the US National Institute of Health[40]. The rationale of this project is that some human genetic polymorphisms have a greater than average influence on the susceptibility to adverse effects of environmental agents. The initial emphasis of the EGP was the selection of candidate genes likely to play an important role in the biologic action and disposal of environmental agents within the human body. Such genes were referred to as environmental response genes. Polymorphisms in these genes are searched for by extensive DNA resequencing. Special attention is given to single nucleotide polymorphisms (SNPs) which occur at about every 1000 nucleotide bases in the DNA sequence. SNPs will be used as signposts in the search for neighboring genes that are involved in environmental response.

Another aspect of the EGP is the study of comparative mouse genomics by a consortium of several research centers. The aim of this work is the development of transgenic (knock-in) mice that are engineered to carry the DNA of a polymorphism for an environmental response gene. Knock-out mice involving deletion of mouse genes that are analogous to human environmental response genes will also be studied.

The human genome project aims to elucidate the standard human DNA sequence with no particular attention to variation. In contrast, research in pharmacogenomics and ecogenomics focuses on variation to select a minority of individuals who may be at risk for drug or environmental damage. Finding such persons requires studying a large number of individuals by investigative approaches such as the search for candidate genes. Once the relevant polymorphism has been detected in a population, search for the specific polymorphism in individuals could be done to avoid potential damage in such persons. The ultimate aim of all such work is to protect individuals at risk.

As in pharmacogenetics, interaction of several genetic polymorphisms relevant to environmental damage may place a person at risk. Careful planning and execution of both the genetic and environmentally aspects of these studies together with sophisticated data analysis will be required.

Emerging data suggest that polymorphic genes of relatively high frequency with low penetrance may often be involved such as in some cancers. This means that a given polymorphism may only manifest occasionally with clinical symptoms thereby making it difficult to elucidate genotype-phenotype correlations.

Population differences in Genetic Polymorphisms

Even though 99.9% of DNA is identical in all humans, there are abundant data indicating that frequencies of most polymorphisms (including those for pharmacogenetic and ecogenetic traits) differ between human populations of various geographic origins such as from Africa, Europe, and East Asia[41]. Specification of ancestral origin of the donor's DNA will therefore be useful but has sometimes been omitted to avoid potential racial discrimination or stigmatization. Because of the frequency of many non-functional DNA polymorphisms that differ between individuals of widely different ancestral origin, unique clusters of individuals characterized by common genetic ancestry can be defined by multi-locus DNA genotyping[42]. With this method, different DNA markers from many chromosomes are used to genotype groups of individuals of different ancestral geographic origin. Based on the different frequency of markers in the various

groups and with appropriate probability statistics, genetically defined clusters related to geographical origins similar to the current classification as Caucasians, Africans or East Asians emerge[43]. The new method[44] also allows measurement of the degree of admixture e.g. that 20% of the genes of African Americans are of European origin. This multilocus genotyping approach therefore allows assignment of genetic ancestry and does not require a priori racial labeling. It remains yet to be demonstrated whether a large number of DNA markers will allow finer distinctions of genetic origin beyond assignment to broad geographic continental areas. It is likely that this will often be possible[44a].

Nutritional Ecogenetics (Nutrigenetics)[45]

Genetic variability of human biochemical makeup affects most metabolic and cellular processes involved in nutrition. Thus, variable genes may impact nutritional requirements for a variety of nutrients. Chronic diseases may have underlying genetic susceptibility factors that interact with the diet to produce a characteristic disease. The genetic hyperlipidemias that predispose to coronary heart disease are well known examples. The role of genetically variable sensitivity to salt in hypertension requires more data.

There are several rare autosomal recessive diseases affecting nutrition such as phenylketonuria, ornithine transcarbamylase deficiency and hypophosphatemic rickets that are "proofs of concept" for the role of genes in nutrition. For example, in phenylketonuria a defective enzyme raises phenylalanine levels which injure the developing fetal brain and produce mental retardation. It has been postulated that the more frequent heterozygotes for such nutritionally related genetic diseases with only 50% of enzyme activity may be at higher risk to develop medical problems under conditions of metabolic stress, parasitism, or malnutrition. More data are required.

A variety of genetic traits related to nutrition have already been described.

Lactose intolerance – Adult hypolactasia[46]

All human infants possess the intestinal enzyme lactase necessary for lactose absorption. In most human populations, in-

testinal lactase disappears after weaning so that most human adults are "lactose intolerant". A mutation allows persistence of lactose absorption. This mutation presumably has a selective advantage in agricultural societies where cow milk is available for nutrition. In central and northern European populations, most persons possess this mutation in either single or double dose. The mutation for persistence of lactose absorption or lactose tolerance is also common in some nomadic pastoralists in Africa and Arabia. "Lactose Intolerance" is caused by lack of this gene and is inherited as an autosomal recessive trait. The mutation for persistence of lactose absorption or lactose intolerance is also common in some nomadic pastoralists in Africa and Arabia. Persons with lactose intolerance tend to develop flatulence, intestinal discomfort, and diarrhea on exposure to milk and other lactose-containing foods but can tolerate small quantities of milk.

G6Pd Deficiency and Favism[47]

Various proportions of the population from tropical or subtropical countries have mutations affecting the red cell enzyme glucose 6 phosphate dehydrogenase. Such individuals may develop red cell hemolysis on administration of a variety of oxidizing drugs. Eating fava beans can also produce hemolysis.

Aldehyde Dehydrogenase Polymorphism[48]

Alcohol is metabolized to acetaldehyde by alcohol dehydrogenase followed by further hydrolysis by the enzyme aldehyde dehydrogenase. A large fraction of Asian populations, such as in Japan, have a deficiency of this enzyme (aldehyde dehydrogenase II) which leads to higher serum acetaldehyde levels after ingesting alcohol. The resultant flushing of the skin is associated with feeling unwell and makes carriers of the enzyme deficiency less likely to become chronic alcoholics. This genetic variant, therefore, is an "anti-alcoholism" gene[49].

A single gene probably involved in Folic Acid Nutritional Requirements[50]

10-15% of the Caucasian population are homozygotes for a genetic polymorphism TT of the folic acid related enzyme

MTHFR (Methylene Tetrahydrofolate Reductase). These TT individuals have raised levels of potentially harmful homocysteine particularly under conditions of low folate intake or when folate blood levels are marginal. (56) Hyperhomocysteinemia in these individuals can be reduced to normal levels by folic acid administration. It is likely that the nutritional requirements for folic acid in such TT MTHFR homozygotes are higher then those of heterozygotes or individuals not carrying this polymorphism[51]. It had already been shown empirically that at least 60% of neural tube defects could be prevented by increased folic acid intake early in pregnancy. Hyperhomocysteinemia is also related to atherosclerosis in coronary artery disease, stroke and peripheral arterial disease[52] and possibly in a variety of other illnesses.

Dyslipidemia and Coronary Artery Disease[53]

Coronary artery disease in one of the most frequent sources of morbidity and mortality in developed societies. The causes are complex and include genetic susceptibility as well as environmental factors such as high fat diet and smoking. Much of the hereditary predisposition can be ascribed to genetic abnormality of lipids. These include high levels of low density *lipoprotein* (LDL) cholesterol, increased small dense low density *lipoprotein,* increased Lpa, apolipoprotein E4, high triglycerides as well as low levels of LDL receptor activity and low high density lipoprotein (HDL) levels. Currently detailed lipid genetic profiles are not done before initiating lipid reduction therapy. However, our group has recently shown that those affected with LDL receptor defects require more intensive lipid reduction regimes for prevention of progressive coronary artery than those with familial combined hyperlipidemia. (manuscript submitted)

The role of the environment is well demonstrated by the higher mortality in carriers of the monogenic LDL receptor defect (familial hypercholesterolemia) in recent years when high lipid diets, smoking and lack of exercise were common as compared with[54] earlier generations when these unfavorable factors were not operative[54].

Oversell of nutritional genomics[55]

While there is a solid scientific basis for some aspects of nutritional ecogenetics - as discussed, several private companies already are commercializing testing for a variety of enzyme polymorphisms as the basis for nutritional advice and selling nutritional products. Unfortunately, the underlying solid research for such recommendations does not yet exist.

Current Status of Pharmacogenetics and Pharmacogenomics[56]

As many other aspects of the human genome project, the genomic applications of pharmacogenetics have been widely discussed in the media and much has been promised for the future. The impression is often left that drug therapy will be revolutionized by elucidating the molecular basis of disease and by a full understanding of the unique cellular and molecular responses to a given drug with avoidance of adverse reactions. Unlike the "same drug for everyone" philosophy, a personalized medicine is visualized which selects a specific drug tailored for each individual based on genetic makeup and mechanism of disease.

While this ideal scenario is likely to apply to some drugs and some diseases it is unlikely that the pharmacogenomics model will be valid for all drugs. Current developments are evolutionary rather than revolutionary in utilizing our better understanding of genetics and disease for drug development and drug therapy[57].

Most pharmaceutical companies have invested in pharmacogenomic research, although companies such as Roche only spend 5% of their total budget for pharmacogenomics[58]. The business side of pharmaceutical companies is often worried that pharmacogenomic approaches will segment the market by discouraging commercialization of block-buster drugs that can be marketed to everyone. Advocates of pharmacogenomics point out that clinical trials will reduce the number of those who need to be tested and therefore will be less expensive. Since current diagnostic designations for a disease may include cases with different etiology, heterogeneity in the case-mix must always be kept in mind. Careful attention needs to be given to environmental differences (e.g. life style, smoking, alcohol exposure,

and diet) in addition to molecular genetic and other laboratory investigation.

Better understanding of molecular mechanisms of a disease may allow insight into altered pathways that may respond to a drug that is specifically designed to act on such an abnormality. Unfortunately, for most common diseases, the genetic etiology is complex and is likely to involve several genes interacting with the environment. Study of the rare single gene subtypes of a complex disease (that occur in no more than 3-5% of those affected with the disease) often detects a uniquely altered pathway. It may be possible subsequently to find a drug affecting this pathway that may also be effective in the "garden variety" type of the condition.

Better understanding of phenotypic heterogeneity such as expression of the neu/her receptor in 25% of cases with breast cancer allowed the development of an effective drug that specifically acted on the abnormal receptor[59]. Another drug – Gleevec - was uniquely devised to counteract excess tyrosine kinase that caused cell proliferation in chronic myelogeneous leukemia[60].

Considerable effort is being given to use single nucleotide polymorphism (SNPs) as markers for closely linked genes that are involved in adverse reactions or differences in response to a drug or a class of drugs. The underlying rationale (ie. a gene of pharmacogenetic interest being located in tight chromosomal linkage with a SNP) is simple but its application will need more work[61]. Variables such as differences in recombination rates ("hot" or "cold" spots), differences in population history, need for very large sample sizes, allelic heterogeneity between populations and various statistical problems must be considered. Attempts to utilize SNPs together with closely linked haplotypes (hap-maps) are being initiated to overcome problems of sample size but still need proof of concept. Another approach is to study populations with few founders (such as Icelanders or Ashkenazi Jews) on the belief that lesser genetic variation facilitates search for genes involved in complex traits. Much of this work is carried out by commercial companies rather than by academic institutions. Claims of interesting data are already being made but specific findings are rarely available.

Another aspect of the increasing commercialization is the establishment of laboratories that offer testing of various sets of polymorphisms claimed to be related to certain diseases and drug responses. While the polymorphisms are usually based on a single or several studies, a general consensus of validity is often lacking as is governmental control of such testing. Services of this type are not required to obtain FDA permission. It is particularly worrisome that such test batteries are not only offered to physicians (and other health providers) but are increasing advertised directly to the public.

Another concern of commercialization are patents and licensing arrangements that increase the cost of drugs and testing for patients. Both pharmaceutical and biotechnology companies justify high prices of drugs by the huge cost of drug development. Whatever the reason, many patients in the United States who lack health insurance may not be able to benefit from new and useful drugs. Much of the world is not at all able to afford these medicines.

Differences in the frequencies of polymorphism involved in drug response and adverse reactions commonly exist between populations[62]. It therefore will often be medically indicated to know the population (race/ethnic group) origin of subjects for pharmacogenomic and other investigations. As an example, in a recent study 20% of black patients with heart failure were homozygotes for both an alpha and a beta adrenergic receptor as compared with 3% of black controls[63]. Caucasian individuals had a very low frequency of these enzyme variants. This information has a solid pathophysiologic basis and requires preventative and therapeutic clinical trials.

New methods of determining racial/ethnic origin (cluster analysis based on frequencies of DNA markers) that do not require identification of race or ethnic origin may have some advantages occasionaly[64]. However, omission of "racial" assignment in individuals who are later identified by the genetic clustering method as of a specific genetic origin may lead to ascribing the study results to hereditary factors while they actually were caused by discrimination or other non-genetic causes such as life style or diet of the group under study[65].

It has been suggested that informed consent for pharmacogenetic tests is different from most other genetic testing since detection for SNP profiles or genes involved in drug response and drug response differs by only searching for a "pharmacogenetic efficiency" or "medicine response" profile. Problems of privacy, confidentiality, stigmatization and discrimination (occupational and insurance) that are usually of concern for genetic testing are said to no longer apply to pharmacogenetic tests that raise no special ethical or social problems. Attempting to find the most appropriate drug for a patient is therefore considered similar to other diagnostic tests in medicine[66]. However, abnormal results are relevant for drug responses in relatives. Other scenarios could be constructed by which those with deviant responses might be discriminated against. Generally, a good case can be made for treating pharmacogenetic tests somewhat differently than more sensitive genetic tests with more serious genetic implications. Recent trends of "genetic exceptionalism"[67] by which any data of potentially genetic relevance are treated separately from other medical or epidemiologic data have been deplored. Instead, it has been maintained that all kinds of medical information should be considered sensitive and only be made available to persons with a true "need to know".

Destruction of DNA specimens that have been collected for pharmacogenetic purposes after initial use generally is not desirable. Novel information is developing rapidly and search for additional polymorphisms often is useful in providing new knowledge and interpretations. Anonymization of DNA specimens is appropriate but retention of information regarding age, gender, ethnic origin, and disease categories will often be helpful. If information regarding racial/ethnic origin is not kept, retesting groups of specimens by cluster methodology could obtain population origin. Contacting original donors of critical specimens for retesting is often logistically impossible.

Education of physicians will be important. Currently, most physicians are poorly informed about genetics and there are not enough medical geneticists and genetic counselors to aid in providing genetic information. However, specialists within the medical profession who are most frequently consulted about genetics such as obstetrician/gynecologists, and pediatricians are gen-

erally better informed about genetics. With the development of cancer genetics and its practical applications, oncologists now are rapidly becoming more knowledgeable about genetics. A potential worrisome development is the increasing employment of genetic counselors by commercial testing companies since it will be difficult for such professionals to be objective about the pro's and cons of testing.

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