Chapter 8 – BIOMARKERS OF INDIVIDUAL SUSCEPTIBILITY

by

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8.1 INTRODUCTION TO GENOTYPES AND BIOMARKERS OF SUSCEPTIBILITY

The biological basis for differences in individual susceptibility to diseases and responses to particular drugs has long been associated with biochemical variations of the same enzyme between individuals [1]. This phenomenon is known as polymorphic variation, and research over the past 60 years has directly linked the sequence and biochemical behavior of variant enzymes to the sequence of the genes encoding them. Such variations in the coding sequences of genes are the essence of individuality. Most of these variations are "neutral," occurring naturally in different populations without detriment to survival and good health. However, certain variations in individual genes or sets of genes can endow individuals with either susceptibility or resistance to disease or to other stressors.

The human genome consists of approximately 3 billion nucleotide sequences distributed among 23 pairs of chromosomes. Only a small percentage of the nucleotide sequences (~3%) encode the proteins essential for human life. The frequency of nucleotide polymorphisms is estimated to be about 1 in 1159 nucleotides in the regions of genes that encode proteins (known as exons). The total number of polymorphisms for enzymes important in the metabolism of drugs and chemicals is not known with certainty, but approximately 50 genes encode human cytochrome P450 enzymes [2]. In one of these genes that encodes an important cytochrome P450 (CYP2D6), over 90 polymorphisms have been discovered to date; some of these appear to be neutral, while others might be beneficial or detrimental in some circumstances [3].

The challenge for geneticists is to identify other relevant variations among the 3 billion nucleotide sequences of the human genome and link them to important phenotypic outcomes such as disease and drug metabolism. The combination of automated sequencing and clone-handling technologies with contemporary bioinformatics tools has made it possible to compare two or more individuals' genomes on a nucleotide by nucleotide basis. One can now find single nucleotide variations between two genomic sequences (single nucleotide polymorphisms or "SNPs"), although such efforts are very laborious. Considering there are approximately 10 million SNPs in the human genome, the task of linking particular SNPs to important phenotypic outcomes is daunting. Fortunately, groups of SNPs tend to be inherited together in blocks of chromosomal DNA called haplotypes. The number of haplotypes ranges from 300,000 to 600,000. By sampling only representative SNPs within a haplotype, the number of genetic markers is greatly reduced to a level that can be practically used in linkage studies to map, locate, and identify genes of importance. A human haplotype map, or HapMap, has been developed by an international consortium and is freely available to the public [4].

8.2 TECHNIQUES FOR DETERMINING GENOTYPES

Early methods of determining an individual's genotype ("genotyping") were based upon various known marker genes in specific regions of the chromosomes. They were laborious to perform and limited with respect to the

numbers of different genes that could be characterized and located. Over the past few years, great advances have produced simplified genotyping technologies using characterized collections of SNPs and haplotypes. A wide range of methods is currently available, and each year new methods are proposed. In general, current methods require extraction and amplification of the desired sequences of DNA followed by detection. The methods of detection include fluorescence, colorimetry, chemiluminescence, mass spectrometry, and others. Many genotyping methods are already commercially available. Table 8-1 lists examples of commercial suppliers and their products for SNP genotyping.

Table 8-1: Suppliers and Commercially-Available Products for SNP Genotyping

Kits	Microarrays
Applied Biosystems • SnaPshot Multiplex Kit • SNPlex Genotyping System • TaqMan SNP Genotyping Assays Beckman Coulter • MegaBACE SNuPe Genotyping Kit • SNPware Reagents Kits Invitrogen • SureScore SNP Genotyping System PerkinElmer • AcycloPrime-FP SNP Kit Promega • READIT SNP Genotyping System Pyrosequencing (Biotage) • Pyro Gold Reagents Sequenom • MassEXTEND Mixes and/or Thermo Sequenase Enzyme Third Wave Molecular Diagnostics • Invader genotyping kits Other Suppliers and Products	Affymetrix • GeneChip HuSNP Probe Array • GeneChip Mapping 100K Array • GeneChip Mapping 10K Array • Par Allele TruTag Arrays GE Healthcare (Amersham Biosciences) • CodeLink SNP Bioarray System • CodeLink Human P450 SNP Bioarray and Reagent Kit Illumina • MHC Exon-Centric Panel • MHC Mapping Panel • Sentrix Human-1 Genotyping BeadChip Custom Arrays Printed by Core Labs
	Other Methods
	SNP genotyping reagents developed in specific labs, i.e. “home-brew”

(Source: Bioinfomatics, LLC, 2005).

Additional technological advances are expected in the near future that will permit rapid, high throughput, and relatively inexpensive SNP genotyping of individuals. These technologies are essential for large-scale studies to establish associations between genetic biomarkers (haplotypes) and diseases or drug response [5]. Most current linkage research tends to be hypothesis-driven rather than discovery research. In the former approach, a set of SNPs (e.g. approximately 10) hypothesized to be important to the outcome variable is selected beforehand (*a priori)*, whereas discovery research uses many different SNPs (e.g. 100,000) and analyzes for associations with outcome variables afterwards (*posteriori*). Hypothesis-driven research can utilize technologies such as mass spectrometry, which can process thousands of samples (individuals) for less than 10 cents per sample. Discovery research is more expensive and requires more high throughput SNP/haplotype testing technology (e.g. microarrays) and greater computational power.

8.3 CLASSIC EXAMPLES OF GENOTYPING FOR OCCUPATIONAL HEALTH

8.3.1 N-Acetyltransferases (NATs)

N-Acetyltransferases (NATs) participate in the metabolism of aromatic and heterocyclic amines including important therapeutic agents. The aromatic and heterocyclic amines are frequently carcinogenic and are constituents of many environmental and occupational exposures. These amines are conjugated through acetylation by NATs and may become either detoxified or, conversely, bioactivated to become more potent carcinogens. It has been demonstrated that NATs have genetic polymorphisms producing enzymes ranging from slow to fast acetylators, which have also been associated with individual susceptibilities to various cancers [6]. The NAT polymorphisms may also predict the toxicity of certain drugs.

There are two prominent NAT isoenzymes (NAT1 and NAT2) encoded by different genes located adjacent to each other on human chromosome 8 [7]. Over the years, both the NAT1 and NAT2 genes have had many polymorphisms identified. Recently, Boukouvala, and Sim [8] conducted a complete structural analysis of the NAT1 and NAT2 genes and characterized the alternative mRNA transcripts. Prior to the capability to identify and characterize the NAT genes and transcripts, a number of probe drugs were used for phenotypic assessment of acetylation capacity in humans [6].

8.3.2 Glutathione S-Transferases (GSTs)

Glutathione S-transferases (GSTs) are important in the phase II metabolism of xenobiotic chemicals. These enzymes, combined with their substrate glutathione, have significant detoxification capacities. Four main classes of human GSTs have been identified: alpha (A), mu (M), pi (P), and theta (T); and each of these classes has one or more isoforms [9]. Polymorphisms in the cytosolic GSTs have been identified in humans and are likely to contribute to the interindividual differences in responses to xenobiotics [10]. Many research studies on the risks of cancer associated with different GST genotypes have serious shortcomings, because they only investigated the effects of homozygous null versions of the genes and did not examine individuals that were heterozygous for null and functional alleles. However, an increased risk of breast cancer was associated with heterozygosity in studies using novel methods, [11].

8.3.3 Glucose-6-Phosphate Dehydrogenase (G6PD)

Perhaps the best-known example of genetic susceptibility to an exposure is the association between erythrocytic G6PD deficiency and an increased risk of hemolytic anemia. G6PD regulates the distribution of glucose-6-phosphate between the glycolytic and shunt pathways to meet cellular needs for reductive biosynthesis and

cellular redox maintenance. Individuals who are deficient in G6PD activity are more likely to experience hemolytic anemia when exposed to fava beans, a number of drugs, and industrial or agricultural chemicals [12]. In the military, the G6PD activity of individuals is often assessed before administering the anti-malarial drug Chloroquine to avoid hemolytic anemia in G6PD-deficient individuals.

The G6PD gene is carried on the X chromosome, and the World Health Organization has classified the hundreds of possible variants into five groups (I – V) of activity in erythrocytes [13]. Progress in the molecular characterization of these variants have been expedited by polymerase chain reaction technology; many of the variants that were once considered distinct based upon biochemical properties have since been determined to be identical at the DNA sequence level [14]. The structural organization of the gene is now known, and recent research suggests that G6PD may play other subtle and important roles in biology, particularly with respect to antioxidant defense.

8.3.4 Paraoxonase (PON1)

Paraoxonase (PON1) is a member of a family of proteins which also include PON2 and PON3. They are serum proteins closely related to high density lipoprotein (HDL). PON1 not only hydrolyzes the active metabolites of some organophosphate pesticides such as chlorpyrifos and diazoxon but also the very toxic organophosphorous agents such as sarin, soman, and Vx [15 – 17]. Plasma PON1 activity in humans is genetically determined and has been found to vary 40-fold between individuals [18 – 20]. Furthermore, the gene frequencies for high or low metabolizers vary among groups with different ethnic and geographical origins [15]. To date, more than 160 polymorphisms have been described for these proteins. People with polymorphisms that debilitate PON1 activity and that limit AChE production are at greater risk for Parkinson's disease when exposed to agricultural insecticides [21].

8.4 THE FUTURE OF GENOTYPING IN MEDICINE

In the past, the greatest advances in medical genetics have been in identifying diseases that are associated with mutations in a single gene. Examples of these simple Mendelian diseases include Tay-Sachs disease and cystic fibrosis [22]. While discoveries of medically important variations within a single gene will continue, many heritable diseases clearly have more complex origins involving variations and interactions between multiple genes and environmental factors [23]. Deciphering these interactions to understand the genetic basis of disease will require special analytical tools and extensive research. Nonetheless, the journey to personalized medicine through genomics and genotyping is well underway.

In the near future, the impact of genotyping upon health care will likely be applications in diagnostics and drug development. Among patients who are prescribed a drug for a medical condition, a certain percentage will either not respond to the drug or will have an adverse reaction. If these patients can be identified by a genetic biomarker, then they can receive an alternative drug or treatment. Already, patients are receiving treatments for cancer on the basis of their genotypes, and more personalized treatments are anticipated. [24]. This type of personalized medicine is being spearheaded by the pharmaceutical industry, which can create new customized drugs and keep existing drugs on the market by identifying subpopulations for whom a drug is beneficial, detrimental, or ineffective.

Along with the HapMap – which will help identify and test for important genotypes – other technological advances will also make genotype screening more feasible. It is predicted that within a decade, the cost of sequencing the genome of an individual will cost $1,000 or less [25]. The implication of this achievement is that a patient's genome sequence will become part of the standard medical record for use in diagnosis,

treatment, and prognosis. For the military, the implications are even greater, because the medical records of military personnel tend to be better documented and computerized compared with civilian medical records, and military personnel are often prescribed mission-related prophylactic drugs and vaccines.

8.5 EPIGENETICS AND ENVIRONMENTAL EXPOSURES

The debate over "nature versus nurture" has existed throughout much of history and has been phrased in terms of science as "genes versus environment." However, recent evidence suggests that the relationship between genes and environment is not dichotomous but rather has a gene-environment interaction [26]. In a recent study conducted with 80 identical twins in Spain, it was determined that the differences in the regulation of gene expression between the twins became greater with the increasing differences in their environments (social and physical) [27]. Such differences in gene expression do not result from alterations in the DNA sequence, but from persistent transcriptional regulatory effects on gene expression. Environmental stimuli giving rise to such effects are called epigenetic factors and can result in phenotypic variation. Several mechanisms have been identified at the biomolecular level that account for epigenetic effects on gene expression, including DNA methylation and histone acetylation. These epigenetic modifications can be heritable through many cell generations [28].

The epigenetic phenomena complicate the application of genotyping in medicine and environmental health. Prior exposures and experiences could conceivably alter the onset and progression of environmentally-induced diseases. Epigenetic phenomena are still relatively poorly understood but may partly explain the differences in responses to environmental stress experienced by genetically similar individuals.

8.6 POTENTIAL GENOTYPING APPLICATIONS IN MILITARY POPULATIONS EXPOSED TO TOXIC CHEMICALS

Over the next two decades, the practice of genotyping will increasingly become part of mainstream medicine as more knowledge is acquired about clinically relevant polymorphisms and genotypes, and as patient care benefits are proven through research. Many of the civilian applications of genotyping will easily transition into military medicine. However, because military personnel face many environmental health hazards and receive medications and vaccines different from the civilian populace, genotype screening to protect troops from these unique exposures will present additional challenges for developing prevention strategies and doctrine on how to use this new knowledge and technology.

The military has the necessary infrastructure and accountability for its patient population to implement genotype screening on a large scale. But the military medical community has yet to develop policy or clinical practice guidelines for genotype screening. An exception to this generality is the use of G6PD screening of military personnel prior to administering anti-malarial prophylaxis. The revolution in pharmcogenomic medicine is coming, and it would be prudent for the military to proactively plan for it. Part of the planning process should include population-based and laboratory research to determine the underlying genetics of health and fitness traits that are relevant to military medicine.

Acknowledgement: We would like to thank Dr. David A. Jackson for his review and comments on this chapter to the report.

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