



The Pathologist's Message

SNPs (Single Nucleotide Polymorphisms) refer to DNA sequence variations in the genetic sequence. For a sequence variation to be considered as a SNP, it must occur in at least 1% of the population. These minor variations from the “standard” or “wild-type” sequence when associated with a particular condition, can serve as a predictor of that disorder. SNPs themselves do not cause disease, but rather can be used to determine the likelihood that someone will develop a particular illness because SNPs can often be located near a gene that causes disease. So far, more than 12 million SNPs have been catalogued.¹ SNPs are a fundamental tool utilized in furthering personalized medicine in two key areas:

- Genetic sensitivity to specific medication: Understanding whether a patient is likely to benefit or suffer from a particular drug will lead to more targeted medications and better patient care.
- Predisposition to future disease: Knowledge of an increased propensity for a specific disease may have significant impact upon lifestyle, medical oversight, and treatment options.

SNP profiling will likely impact the practice of pathology. As the understanding of drug metabolism expands and the fundamental role of population subgroups becomes more evident, prescribing physicians will incorporate SNP profiling results in their pharmacologic treatment regimen. SNPs that are predictive of altered drug responses have already become part of the qualification criteria for patients to receive particular medications, especially if the treatment is expensive, toxic, or has a narrow therapeutic index. For example, SNP testing for warfarin sensitivity identifies certain genetic variants that are clearly associated with over-anticoagulation at standard doses. Warfarin is the most commonly prescribed anticoagulant for prevention of thromboembolism, with more than two million patients in the US alone. Warfarin also accounts for more than 15% of all severe drug-induced adverse events, much of which is due to SNP variations in the *CYP2C9* gene sequence.²

As SNPs become increasingly associated with disorders and genetic predisposition, pathologists will directly use SNPs as another element in their diagnostic armamentarium, thereby directly influencing treatment planning. Adequate training to properly interpret and integrate results will be necessary.

Ultimately, consumer and physician demand for this testing will depend on the value of the information provided. For certain drugs and diseases, SNPs may have significant utility. As the library of SNPs and associated drug responses or diseases grows, demand will increase. Although relatively few SNPs may be diagnostic, many are likely to be included among the aggregate mass of evidence to support a particular diagnosis and patient treatment plan recommendations.

Regardless of whether the laboratory is conducting the tests, the local pathologist should be knowledgeable about SNP profiling and its implications in diagnosis and treatment. SNP tests will be ordered both by pathologists and local providers, and the pathologist will need to guide the immediate caregiver to determine how the information may affect the diagnosis or treatment plan.

Specifically, the pathologist has three distinct roles.

1. For labs that have sufficient volumes to provide nucleic acid analysis, the pathologist will utilize molecular techniques for SNP analysis to provide the primary data.
2. Whether the technical analysis is performed in the local pathologist's laboratory or sent out to a reference laboratory, the pathologist needs to interpret the technical result in the context of an individual patient's overall medical condition. In this way, SNPs are analogous to many other laboratory tests that have distinct technical and professional components. The pathologist should include their findings as a part of the interpretive report including the specific patient's genetic basis for drug selection or disease status.
3. For laboratories with insufficient volumes to justify in-house testing, the pathologist should provide guidance in the selection of reference laboratories to conduct this testing.

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Clinical Context

Pharmacogenomics focuses on examining the genetic basis for individual variation in response to a given drug, with the goal of reducing the guesswork in prescribing medications and increasing the likelihood that the right drug is given to the right person at the start of therapy. SNPs in the genetic code may or may not be significant enough to interfere with vital biochemical processes. All genes harbor SNPs; however, identification of those that are most relevant remains a challenge. Molecular techniques are used to identify SNPs relevant to drug efficacy or associated with responses. When SNPs are identified in the portion of the genetic sequence associated with drug metabolism or action, those variations can affect the effectiveness of a particular drug.

For example, SNPs associated with the *CYP2C9* or *VKORC1* genes occur at a location that results in a protein with diminished function compared to what would have been produced by a “normal” (*i.e.*, “wild type”) gene with the “normal” (*i.e.*, non-SNP) sequence code. Patients with these mutations are significantly more sensitive to the action of warfarin than “normal” patients. This result helps explain the 10-fold interindividual variation in warfarin dosing. In these patients, typical starting doses of warfarin result in far greater INR (Prothrombin Time International Normalized Ratio) values than are to be expected in the general population. Consequently, the patient is at an elevated risk of stroke or hemorrhage until the circulating drug is cleared and the daily dosage is reduced. This outcome is particularly true for a drug like warfarin, which can take 5-7 days for full clinical effect in a “normal” person and thus efficacy testing is typically delayed for weeks after initiation of treatment. In effect, the patient is “overdosed” with a “normal” dose.

According to the FDA's Adverse Event Reporting System, warfarin is among the top 10 drugs with the largest number of serious adverse event reports. US death certificate data for 2003-04 indicate that anticoagulants are cited most frequently for deaths related to adverse drug events. Hospital emergency department data between 1999-2003 list warfarin second amongst all Emergency Department visits for prescription drugs adverse events.³ As a result, the FDA announced in August 2007 that the labeling for warfarin was to be updated to include guidance on administration based on pharmacogenetic information (*CYP2C9* and *VKORC1*).⁴

Overall, such adverse drug reactions (ADRs) account for up to 7% of US hospitalizations and are estimated to be the 5th or 6th most common cause of illness and death in the US.

The annual cost of ADRs is in the range of \$30-\$150 billion, primarily due to prescription error, overdosing, drug interactions, and population genetic variables. When coupled with the cost of new drug developmental cost (\$500-\$700 million) and limited time to recover these costs while under the remaining patent life (5-7 years), the pharmaceutical industry is increasingly interested in preventing ADRs while simultaneously providing the drugs to the maximum number of patients likely to see a benefit. At present, only a limited number of SNPs have been identified to segregate groups of patients into responders, nonresponders, and adverse reactors to a given medication and dose.

Alternatively, SNPs identified in other regions of the genetic code are utilized quite differently. Since less than 5% of the human DNA sequence actually codes for protein production, most SNPs are identified outside the coding sequences. As such, they are simply local differences that may or may not have a direct effect upon a disease process. Nevertheless, they are heritable, and thus may be useful for identifying genetic disorders that predominate among genetically-related populations. In other words, SNPs are in linkage disequilibrium with disease genes. For example, if a particular SNP were to be mathematically associated with a 40% increase in colon cancer, then that SNP may be recognized as a risk factor for colon cancer. However, that biomarker (SNP) need not be located in any portion of a genetic sequence directly related to the disease state (colon cancer). It may simply be coincidental that the biomarker was more frequent in a specific population that had an increased prevalence for the disease.

For example, the ApoE gene codes for a protein associated with lipid metabolism. In the general population, there are 3 variations caused by SNPs at positions 112 and 158. (See Table below.) The effectiveness of lipid-lowering agents such as statins depends upon which ApoE variation is present. However, one of the ApoE gene variations (E4) has also been associated with late onset Alzheimer's Disease (AD) in symptomatic adults. The variation is not diagnostic of Alzheimer's, but in a patient with dementia, the presence of that ApoE variation increases the likelihood that the dementia is due to AD. Increasingly, direct-to-consumer laboratories are marketing tests such as the ApoE SNP as a predictor of future AD. Examples of other genetically identifiable diseases via SNPs are cystic fibrosis, hemochromatosis, and certain infectious diseases such as MRSA bacteremia.

<i>ApoE</i>	<i>Position 112</i>	<i>Position 158</i>	<i>AD Risk</i>
E2	Cysteine	Cysteine	Average
E3	Cysteine	Arginine	
E4	Arginine	Arginine	Higher

Clinical Context (Continued)

The potential uses of SNPs both in the management of current and prediction of future disease are generating considerable excitement in many corners of the healthcare delivery system. Genetic diversity, most notably through SNPs and copy-number variation, together with specific environmental exposures, contributes to both disease susceptibility and drug response variability. SNP identification will be increasingly utilized for determination and prediction of genetic disorders and associated diseases, as well as for prediction of favorable drug response. As identification and clinical understanding of genetic variants grow, pathologists will have new data to contribute to each patient's diagnosis and treatment plan.

Technology Overview

A SNP occurs when a specific position in the gene sequence has a different nucleotide (A, C, G, or T) than present in the "normal" (*i.e.*, "wild-type") population. The variation may or may not change the coded amino acid or directly affect the biological function, but may be statistically associated with some defined condition such as altered drug metabolism or disease predisposition. SNPs occur in the human population relatively frequently, with every individual carrying a unique assortment.

SNPs occur throughout the genome and tend to be relatively stable genetically. Therefore, they can function as excellent surrogate biologic markers. They can be used to not only identify variations in gene sequences and the consequent proteins, but also they can be mathematically associated with genetically-based traits even if the specific gene(s) or protein(s) are unknown. Novel, high throughput techniques continue to generate enormous databases. At present, there are approximately 12 million SNPs identified in public databases. However, the collateral data regarding the drug metabolism characteristics also needs to be collected. As the databases become more complete, complex software algorithms are being used to identify the critical associations of SNPs and pharmacogenomic information.

Test Methods Used: Relatively few pathologists outside of industry are actively involved in generating databases of SNPs and associated conditions or predispositions. The majority of pathologists, however, will use a variety of proven methods to identify SNPs either as vendor-supplied technology or reference laboratory sendouts.

The typical method utilizes patient DNA (usually from WBCs in a peripheral blood specimen) which is evaluated for SNPs by SNP-specific nucleic acid probes that hybridize to specific DNA sequences. Detecting that hybridization depends upon a signal being generated by chemical reactions.

Acceleration/Deceleration Triggers to Adoption: SNPs and other similar technologies ultimately are market-driven toward clinical applications to predict drug effectiveness or diagnose diseases. For some diseases, such as cystic fibrosis, the common mutations are well-understood. However, for other diseases, such as cardiovascular disease, diabetes, or certain cancers, the underlying complex genetics and numerous confounding multifactorial environmental variables complicate the clinical decision-making, effectively magnifying the difficult translation of SNP technologies into the clinic. Many SNPs are insufficiently validated and can be misleading to apply across genetically distinct populations. In still other areas, the link between genotype and phenotype needs to be better understood before widespread clinical adoption.

Most vendors use some variation of proprietary probes and chemistry to identify SNPs. Vendor offerings also differ by assay technique, range of SNPs detected and use of available SNP database libraries.

For example, Nanosphere's Verigene system uses gold nanoparticles to identify hybridized complexes formed between sample patient DNA and defined oligonucleotides in a self-contained, single-use test cartridge. This system is capable of genetically identifying cystic fibrosis, hemochromatosis, and thrombosis predisposition in a point-of-care platform.

Today, the business of SNP analysis is largely in the direct-to-consumer arena. Most often, these single technology laboratories market directly to the end consumer and provide tests results with little interpretation (e.g., 23AndMe, Navigenics Sequenome, DeCodeMe).

Impact on Current Pathology Practice: SNPs provide pathologists with another tool for improving prescription drug selection, efficacy, and safety, and providing additional data to determine genetic propensity for disease, potentially permitting earlier intervention. Overall, the impact on pathology practice will be evolutionary, not disruptive.

Pathologists are in the best position to integrate the SNP profiling results with the clinical information of the given patient. Regardless of where the test is performed, they will be called upon by their peers to understand the nature of these tests and interpret the results. The pathologist's role is to ensure the quality of testing and the clinical relevance of SNP variants. Consequently, the pathologist will help set highly ethical standards on the appropriateness and clinical applicability of SNP testing. If sufficient volume exists for conducting in-house testing, SNP profiling could produce an additional revenue stream.

The end consumer market is driving SNPs, especially for genetic predisposition to diseases such as cancer. Some SNPs will be necessary for diagnosing specific diseases and drug responses that have a proven value, but that quantity is still unclear. Also unclear is whether there is sufficient volume for SNPs to be performed by groups other than reference facilities. The key barriers to adoption of SNP profiling currently centers on the availability of useful data for interpretation that has a demonstrable impact on the ultimate outcome.

Pharmaceutical companies and patient safety advocates are driving the use of SNPs for drug selection. However, if pharmacogenomics only produces a narrow range of SNP-targeted diagnostics and therapeutics, there may be little financial incentive to drive its widespread use in clinical practice or health promotion. In addition, if clinical correlation data based on biochemistry, physiology, or symptomatic findings prove to be as meaningful as SNP data in tailoring therapies, the drive for adopting pharmacogenomic advances may diminish.

The potential applications of pharmacogenomics are generating considerable excitement in many corners of the healthcare delivery system. However, much remains to be determined before its promises can be realized. At present, there is little published data on the clinical effects of genetic polymorphisms on drug targets, drug activity, and biological and environmental factors that influence clinical outcomes. Very few prospective, genotype-guided treatment selection trials are described which positively impact the patient's survival or clinical course. Furthermore, what is currently known pertains mostly to monogenic defects that produce either distinct phenotypes in rare individuals or easily measurable polymorphic phenotypes. The ability to investigate complex, polygenically determined phenotypes relating to drug efficacy/toxicity or disease predisposition is in its infancy. As identification and clinical understanding of genetic variants associated with drug response grow, pathology will have new tools to refine each patient's diagnosis and customize their treatment.

As the inclusion of SNPs for pharmacogenomics and disease prediction increases and becomes more applicable to more patients, practitioner responsibilities will increase. Providers already incur difficulties in identifying potential genetic aspects of their patients' diseases and in appropriately referring them for counseling or treatment. Features of genetic disorders, such as incidence, penetration, variability, implications for blood relatives, and patterns of natural history, are unfamiliar to many established providers. Thus, getting physicians to think "genetically" poses challenges that are wholly different from adopting a new diagnostic or predictive tool.

The ability and willingness of pathologists to adopt diagnostic innovation into their practice will be a key factor in assisting those who decide to think "genetically".

For More Information/References:

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