Upon successful completion of this continuing education program, the pharmacist should be able to:
1. Describe the opportunities for the application of pharmacogenomics in pharmacy practice.
2. List and explain pharmacogenetic tests used for guiding drug choice and/or doses of specific drugs.
3. Identify gene-drug (biomarker-drug) pairs with pharmacogenomic applications.
4. Compare and contrast the methods of DNA sample collection and how this can be incorporated into pharmacy practice.
5. Identify, based on the patient’s medication profile, which pharmacogenomic tests should be performed.
6. Interpret and apply pharmacogenomic test results.

Upon successful completion of this activity the pharmacy technician will be able to:
1. Describe the opportunities for the application of pharmacogenomics in pharmacy practice
2. List and explain pharmacogenomic tests used for guiding drug choice and/or doses of specific drugs.
3. Identify drugs commonly stocked in a community pharmacy for which genetic testing exists and refer patients to a pharmacist for further assessment.
4. Compare and contrast the methods of DNA sample collection and how sample collection fit in pharmacy workflow.

INTRODUCTION
Pharmacogenomics (PGx) is a new frontier for health care that has the potential to transform medical practice and offers pharmacists opportunities for increased value recognition. The traditional approach to medication prescribing is treating patients in a “one size fits all” approach, based on results from highly controlled clinical trials. However, even with these trials, the Food and Drug Administration (FDA) requires post-marketing surveillance to more fully understand how people respond to the medications. Even with all of this, patients experience adverse events from medications recently estimated to cost $200 billion annually. The health care community generally knows that side effects will occur and that people may respond differently to medications, but until now, we have not been able to identify and predict these variations before the patient takes the medication. Understanding and applying genetic knowledge of how people differ can enable health care professionals to provide tailored and more targeted therapy, enabling patients to have better outcomes with fewer adverse effects and lower health care costs.

For pharmacists to remain the “medication experts,” they must take it upon themselves to incorporate PGx into their practices. Doing so provides opportunities to differentiate pharmacists’ value and establish new compensation mechanisms. With PGx, pharmacists can improve patient safety and help patients have better therapeutic outcomes through targeted application of medications. Pharmacists need to understand the basics of PGx, what tests are indicated, how tests are performed, and how to interpret and communicate the results to both patients and other health care providers.

OVERVIEW
Since the early 1990s, the pharmacy profession has been
continuously identifying ways to establish its value and increase recognition for helping patients use medications correctly. Pharmacy has sought to grow from a focus on the product to a service-oriented approach of “pharmaceutical care” and now “medication therapy management (MTM).” Within this effort, pharmacists have also been trying to establish opportunities to be recognized and paid for these services which improve the outcomes of medication use. In the 20-plus years of this movement, the focus has been on disease states that have high costs to the health care system (such as diabetes, high cholesterol and cardiovascular disease, and asthma) and incorporating wellness and lab testing into pharmacies with programs such as cholesterol testing, bone density testing, and immunizations, to name a few. Pharmacist services have also expanded through the implementation of Medicare Part D and the MTM program requirement. Still, implementation of many of these programs has been difficult as patient opportunities and compensation have been sporadic, leaving pharmacists still seeking more formal recognition through efforts such as ‘provider status.’

One of the most successful of these programs has been immunizations. Pharmacists have been able to successfully incorporate immunization delivery into their practices, providing a mechanism to extend their practices and service revenue opportunities. It is now commonplace for patients to expect to be able to walk into a pharmacy and receive an influenza vaccination in the fall or to even receive pneumococcal or HPV (human papillomavirus) vaccinations. This service has most likely been successful compared to other types of services for three reasons: providing the vaccination has been effectively inserted into pharmacy workflows, the service increases convenience for patients, and pharmacies have been able to fit into the compensation model and be reimbursed for vaccine product and vaccine administration. Pharmacogenomics has the potential to fit into pharmacy practice in much the same way.

UNDERSTANDING THE SCIENCE

Mapping of the human genome began in 1990, with the announcement of the production of the human genome sequence in June of 2000. A draft of the human genome sequence was published simultaneously in Science and the journal Nature in February of 2001, with the project being formally completed in 2003, two years ahead of schedule. Public and private efforts were undertaken simultaneously, with the public project being coordinated by the National Institutes of Health (NIH) and the Department of Energy. Much of the work was done in universities across the United States and other contributors participated from around the world. The effort was to sequence all of the three billion bases of deoxyribonucleic acid (DNA) within the entire human genome and to find all of the estimated 20,000 to 25,000 human genes. Since the project’s completion, researchers have sought to understand its implications of and applications for this new knowledge.

One application for the knowledge gained from the human genome project is in the area of health care. Genomic tests have been developed to identify if a person is at-risk for certain diseases, if parents are carriers for genetic disorders that may make them more likely to pass a disease to a child, and to predict responses, both therapeutic and adverse, to drug treatment. This latter case is the aspect of PGx and is where pharmacists can apply this science to their patients and work with physicians and patients to improve patient care specific to medication use. By understanding how people’s genes differ, we are now able to understand how individuals may respond differently to drug therapy. Patients are typically categorized based on their rate of metabolism, known as metabolizer phenotypes, as can be seen in Table 1.

These variations in metabolism can result in different responses to medications. Someone who is an ultrarapid metabolizer (UM) of an active medication is likely to have lower concentrations of the drug and may be considered a treatment failure, whereas someone who is a poor metabolizer (PM) may have higher than expected drug concentrations resulting in toxic-
Consequently, someone who is a UM would require a higher dose of a drug to get the expected results from a medication as their body eliminates the drug faster. An individual who is a PM would require a lower dose of a drug to achieve therapeutic concentrations. Knowing the patient’s genetic profile and related metabolic phenotype in advance of treatment can aid in helping the patient avoid these undesirable situations and enable the clinician to treat the patient more effectively.

UNDERSTANDING THE NOMENCLATURE

One of the challenges of applying PGx is understanding the terms used. Table 2 contains a list of some basic terms that the pharmacist applying pharmacogenomics in practice will need to learn and be able to use.

Many gene-drug interactions are related to drug metabolism and specifically to metabolizing enzymes of the cytochrome P450 Family (CYP450). Pharmacists will need to be able to communicate these terms as well. Table 3 breaks down the cytochrome P450 terms to enable pharmacists to communicate using them.

What causes these variations that result in patients’ altered metabolism? Most commonly, single nucleotide polymorphisms (SNPs; pronounced “snips”), which are single changes in a DNA sequence. Deoxyribonucleic acid is comprised of four “bases” adenine (A), cytosine (C), guanine (G), and thymine (T). The sequence of bases, in what is called the “coding region” of a gene, is responsible for generation of amino acid sequences which form proteins. The proteins of interest in pharmacogenomics are drug receptors, drug metabolizing enzymes, such as the previously mentioned CYP450 Family, and drug transporters. A change of one base to another in the DNA sequence can alter the amino acid sequence, which in turn may alter protein function. For example, part of the common base sequence may be AAGGCTAA, while an individual may have a variant base sequence of AAGGTTAA, where thymine (T) replaces cytosine (C). These single substitutions are what most commonly introduce variation in gene expression (protein synthesis) and consequently an individual’s response to drug therapy. Not all variations are necessarily labeled SNPs and not all variations cause clinical differences. First, to be labeled a SNP, a variation must occur in at least 1 percent of the population. Second, SNPs in the coding regions of DNA have a greater influence on proteins of clinical import than SNPs in non-coding regions.

SNPs make up about 90 percent of all human genetic

| Table 2. Some Basic Terms and Definitions Pharmacists Use Relative to Pharmacogenomics |
|----------------------------------------|-------------------------------------------------|
| Term                                   | Definition                                                                                       |
| Single Nucleotide Polymorphism (SNP)   | A variant DNA sequence in which a single nucleotide is replaced by another base, such as cytosine (C) replacing thymine (T). |
| Allele                                 | An alternative form of a gene inherited from each parent. One of two or more alternative forms of a gene. |
| Heterozygous                           | Possessing two different alleles for the same trait.                                               |
| Homozygous                             | Possessing identical alleles for the same trait.                                                    |
| Pharmacogenomics                       | The study and application of genetics in the use of medications and response to drug therapy.      |
| Reference Sequence (rs) Number         | A specific number assigned to each SNP providing a unique reference number for each SNP. (e.g. rs123456789). This system is used for the Single Nucleotide Polymorphism Database (dbSNP) hosted by the NCBI (National Center for Biotechnology Information). |

<table>
<thead>
<tr>
<th>Table 3. Explanation of Cytochrome P450 Naming Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>Superfamily</td>
</tr>
<tr>
<td>Family</td>
</tr>
<tr>
<td>Subfamily</td>
</tr>
<tr>
<td>Individual Member</td>
</tr>
<tr>
<td>Star</td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>Suballele</td>
</tr>
</tbody>
</table>
variations and are seen every 100-300 bases along the 3 billion base human genome sequence. Therefore, SNPs can influence everything from individual characteristics to disease susceptibility to drug response.

**MECHANISMS OF ALTERED DRUG RESPONSE**

Pharmacists are accustomed to understanding drug dynamics and kinetics. Single nucleotide polymorphisms primarily affect drug response in three ways that relate to these concepts. By causing altered drug receptor sites, altered metabolizing enzymes, and altered cell transporter mechanisms, single nucleotide polymorphisms can affect patients’ response to drug therapy in multiple ways.

**DRUG RECEPTOR ALTERATIONS**

Single nucleotide polymorphisms in drug receptor genes can result in altered activity of a given receptor and can also change how drugs interact with or bind to the target receptor. Also, some genetic variations include altered production (expression) of a given receptor. Therefore, the pharmacodynamics of a given drug can be altered by a SNP. For example, a SNP in the coding region, as part of other variations, in one protein subunit of the vitamin K epoxide reductase complex (VKORC) results in decreased production of this subunit, known as VKORC1. As VKORC is responsible for reducing vitamin K to its active form and as VKORC is inhibited warfarin, a variant form of subunit VKORC1 results in lower amounts of the enzyme and therefore individuals with this variant require lower doses of warfarin. Lower doses are required because these patients are producing less reduced (active) vitamin K than patients with normal levels of VKORC1 making them more sensitive to warfarin and hence, for patients with this specific genetic alteration, warfarin is more “potent” as their pharmacodynamics have been altered.

**DRUG METABOLIZING ENZYME ALTERATIONS**

Single nucleotide polymorphisms in drug metabolizing enzyme genes can influence the clearance and the half-life of drugs, which impacts maintenance dose requirements and dosing intervals, respectively. Consider the gene-drug interaction of CYP2C19 and citalopram. In CYP2C19 PM (see Table 1), the clearance of citalopram is decreased, resulting in an increased area under the citalopram concentration-versus-time curve of greater than 100 percent. This increased exposure to citalopram can result in the serious arrhythmia known as torsades de pontes, which is life-threatening. This specific gene-drug interaction is discussed further on page 58.

In general, the concentration versus time data for a given drug in patients across the metabolizing phenotype spectrum is predictable with PM having the largest area under the curve (AUC) and IM, EM, and UM individuals having progressively lower AUCs (see Figure 1).

The consequences of a decreased clearance include higher peak concentrations and the requirement of a lower maintenance dose to provide therapeutic concentrations and decrease the risk of toxicity. A lower clearance also results in a longer half-life requiring a decreased dosing frequency to avoid drug accumulation and toxicity.

It must be recognized that a variant gene resulting in altered drug metabolism could have an effect on the conversion of a prodrug to its active form. For instance, the prodrug codeine is converted to morphine, which is mainly responsible for the analgesic effects. Individuals who are CYP2D6 PM do not readily convert
codeine to morphine, and likely do not experience analgesic benefit from receiving codeine. Conversely, CYP2D6 UM convert excessive amount of codeine to morphine and have increased risk of opioid toxicity. Figure 2 presents concentration versus time data for active drug derived from a prodrug in a PM and a UM.

**DRUG TRANSPORTER ALTERATIONS**

Drug Transporters are responsible for the absorption, distribution, and elimination of many drugs. They are present in many different cells/tissues/organisms of the body, including but not limited to the intestines, liver, and kidneys. Because of this, they impact all aspects of medication effectiveness. Transporters are grouped into two major categories: influx (uptake) and efflux transporters. These transporters are responsible for the influx and efflux (in and out) of drugs between various systems of the body (such as intestine to blood, blood to bile and urinary excretion).

As an example, P-glycoprotein (P-gp) is an efflux transporter that moves certain substrate drugs out of cells. The gene that codes for P-gp, ABCB1 (ATP-binding cassette B1), also known as MDR1, has been shown to have variant forms (such as, rs1045642). In the intestine, P-gp works to move drug from intestinal epithelial cells back into the gastrointestinal lumen. This would result in a decrease in bioavailability for P-gp substrate drugs. P-glycoprotein in the brain would move drug from the brain to the blood, thereby potentially decreasing the effect of centrally acting drugs.

A second example of the effect of transporter genetic variation on pharmacokinetics and pharmacodynamics is that of SLCO1B1, which is a gene coding for the organic anion transporter polypeptide 1 (OATP1B1). OATP1B1 is an influx transporter found in various tissues. The *5 variant (rs4149056) has decreased activity and does not move as much drug into cells/tissues. An individual with the SLCO1B1*5 variant would likely have higher concentrations of drug in the blood with resultant pharmacokinetic parameters being altered. Here, the volume of distribution would be smaller as drug is not being moved as readily into tissues. If a given drug is metabolized in the liver, the *5 variant would result in less drug being moved into hepatocytes, resulting in decreased drug clearance.

When considering transporters, the effect on bioavailability relative to influx and efflux of drug molecules changes relative to the tissue in which the transporter is expressed. In the gastrointestinal track (epithelium), efflux transporters work to decrease drug absorption and bioavailability. In contrast, influx transporters can aid in drug absorption and increase bioavailability. Conversely, efflux transporters in the renal proximal tubules (luminal membrane) facilitate drug excretion whereas as influx transporters can influence drug reabsorption. As transporters are identified, their influence on pharmacokinetics and drug dosing needs to be understood. When genetic variance is introduced, the consequences of altered transporter function must be taken into account.

**PHARMACOGENOMIC TESTS**

Currently, pharmacogenomic tests are typically ordered for specific gene-drug interaction analysis. Here, testing is ordered because a specific drug is intended to be used and there are known genetic variants related to the drug receptor, transporter, and/or metabolizing enzyme. Table 4 provides a list of the pharmacogenomic tests for cytochrome P450 genes and variants and the rationale for the given test.

**COLLECTING THE SAMPLE**

One of the reasons pharmacogenomic testing can fit into pharmacy practice so well is that samples can be collected very easily. There are three main sources of DNA...
for testing, including blood, saliva and buccal cheek cells. While blood can be ideal, saliva and buccal cheek cells are sufficient mediums and are much easier to collect. While both saliva and cheek samples can be collected by a patient, patients may choose to have this performed by a health care professional. With the buccal cheek swab procedure, a kit is used that has a cotton tip or foam sponge tip that looks similar to a cotton swab. Typically, the swab is inserted into the mouth and rubbed firmly against the inside of the cheek, between the gum and cheek or between the gum and the lower and upper lip.

Commonly for collection, the cheek is rubbed for approximately one minute. The swab is then placed in a tube or envelope for storage and shipping to the laboratory for processing. With the saliva collection kit, patients simply spit into the test tube to provide enough saliva (approximately 5 mL) for testing. The volume of saliva can seem large to patients and it may be difficult for some patients to supply the needed volume. It may take five to ten minutes to collect the required volume. Once collected, the test tube is sealed and sent for testing. With both collection methods, it is important that proper technique is followed so that there is no contamination with another individual’s DNA. If there is contamination of the sample, or if not enough sample is collected, the patient may be required to provide another sample, delaying results and increasing costs. Still, both of these sample collections are generally easy to perform and in some cases are marketed directly to consumers for collection in the home.

Some experience has shown that failure rates when consumers are collecting on their own can be as high as 10 percent. This may be justification for collection being performed by trained health care professionals such as pharmacists.

**AT THE LAB**

While pharmacists most likely will not be involved in performing the actual lab tests with the sample, it will be useful in practice for pharmacists to have an understanding of how the testing is performed. To test an individual relative to a pharmacogenomic gene of interest, DNA must first be purified and isolated from the provided sample. Initially, the cells containing DNA must be lysed to release the DNA and other cellular content. Lysis is typically accomplished by adding a lysis buffer, which typically contains detergents, salts and proteases. As an example, cells can be placed in a hypotonic lysis buffer

<table>
<thead>
<tr>
<th>Test: Alleles tested</th>
<th>Rationale</th>
</tr>
</thead>
</table>
| **CYP2D6:**  
Normal function: *1, *2, *2A,*35  
Gene duplications: *1, *2, *4, *10, *41 | To determine the patients metabolic phenotype for drug and drug dose considerations:  
UM  
EM  
IM  
PM |
| **CYP2C9:**  
Normal function: *1  
Decreased function: *2, *3 | To determine the patients metabolic phenotype for drug and drug dose considerations:  
EM  
IM  
PM |
| **VKORC1:**  
Normal expression: G  
Decreased expression: A | To determine VKORC1 genotype relative to warfarin sensitivity*:  
GG  
AG  
AA |
| **CYP2C19:**  
Normal function: *1  
Loss-of-function: *2, *3  
Gain-of-function: *17 | To determine the patients metabolic phenotype for drug and drug dose considerations:  
UM  
EM  
IM  
PM |

*Utilized with CYP2C9 specifically for warfarin dosing.
which results in the cells swelling and bursting, thus releasing the cellular content. Once released, cellular debris and DNA must be separated, which can be performed by centrifugation and filtering and removes insoluble materials. Using certain reagents and specific matrices, the DNA is precipitated and isolated from other soluble material. Once isolated, the DNA can be prepared for amplification and analysis.

One approach to analyzing a DNA sample is to use polymerase chain reaction (PCR) to amplify the DNA and gel electrophoresis to separate DNA strands by size. The preparation of a sample of DNA for PCR includes making a solution of components that promote DNA amplification. (For a video of DNA PCR, visit http://www.youtube.com/watch?v=2K0LntwoZKU. For an interactive PCR tutorial, visit http://learn.genetics.utah.edu/content/labs/pcr/.)

The components typically include nuclease-free water, a buffer, primers, nucleotides, DNA polymerase, and the DNA sample. The primers are approximately 20 bases in size and are complementary to the DNA (gene) of interest. As DNA is double-stranded, one primer is designed to be complementary to the end of one strand and a second primer is complementary to the other end of the second strand. When the solution is complete, containing all components needed for PCR, the solution is placed in small “PCR tubes” and placed in a thermocycler. Aply named, the thermocycler cycles through periods of heating and cooling. As the solution is heated, the DNA strands separate (denaturation). The primers attach (anneal) to their complimentary DNA from the DNA sample. DNA polymerase works to add the nucleotides to complete the complementary DNA strands (elongation). The thermocycler then decreases the temperature to complete a cycle. The “heating-cooling” cycle is repeated numerous times (as many as 44 cycles). Each time the thermocycler heats the sample, the DNA present separates and the process described above repeats. This cycling process results in the formation of billions of copies of the DNA, thus “amplifying” what was provided in the original DNA sample.

In some protocols, more than two primers may be added to amplify more than one DNA variant at a time. For instance, the following four primers are used in a protocol to identify whether CYP2C19*1 (most common form) or CYP2C19*2 (variant form) are present in a given DNA sample. The primers are short DNA sequences: primer 1-CAGAGCTTGGCATATTGTATC, primer 2-GTAAACACACAACATAGCTATG, primer 3-ATCATTGATTATTCCCA, and primer 4-AATTGTTATGCGTCCC. The primers are included in a solution with nuclease-free water, a buffer, nucleotides, DNA polymerase, and the DNA sample. The thermocycler is programmed for 44 cycles and runs over a time period of approximately 2.5 hours. As DNA from numerous individuals may need to be tested, multiple samples are prepared and placed into individual PCR tubes in the thermocycler. Once the process is completed, each sample of the amplified DNA is placed into a “well” in a gel to undergo electrophoresis. In general, electrophoresis is the process of using electric currents to move molecules.

Gel electrophoresis (http://learn.genetics.utah.edu/content/labs/gel/) is the process by which pieces of DNA are separated, according to size, by passing through a gel with an electric current. An electrophoresis gel is typically composed of agarose, a polysaccharide obtained from seaweed. Agarose is provided in a granule, free flowing powder form which is added to buffer solution and then boiled. A fluorescing agent (such as ethidium bromide) is added to the solution. The hot solution is poured into a mold containing a plastic “comb” which is used to form wells for sample placement. When the solution cools it forms a gel, the comb is removed and the wells available for samples. Each sample with the amplified DNA is placed in a well in the gel within an electrophoresis apparatus. The apparatus has electrodes at each end such that a current passes through the gel from the negative electrode to the positive electrode. The wells, containing the DNA samples, are placed at the negative end of the apparatus. Electrophoresis causes the negatively charged DNA pieces to move away from the negative electrode end of the gel toward the positive electrode end of the gel. The smaller the DNA size, the further it is able to travel through the gel, thus separating different sizes of DNA. Figure 3 presents an image of a gel following approximately 40
minutes of electrophoresis. Notice the DNA is separated by size, with the smaller sizes traveling further through the gel, resulting in separating of the different DNA forms.

The basic PCR and electrophoresis methods described here are meant to introduce, in general, the approach to identifying variants of a single gene as may be applied in many research laboratories. Beyond the PCR approach, other methods of DNA interrogation, with much greater capacity have been developed.

As an example of advanced DNA interrogation, a microarray, also known as a “bio-chip”, is silicon, glass, or other solid material to which DNA probes are attached. The probes, complementary genetic sequences, can interact with a sample of DNA to identify many different genes variants simultaneously or “in parallel.” In fact, each array can contain thousands of probes which allow for determination of genetic variation in multiple genes at the same time. Upon sampling of DNA, with a blood test or buccal swab, the DNA is amplified and labeled typically with a fluorescence compound. The DNA is then hybridized (bound to) the chip where it can interact with the probes. The microarray is then scanned, for instance with a laser, to excite the fluorescent compound and the DNA-probe interactions are identified by the fluorescing labels.

For instance, a particular commercially-available array (larger research institutions will make their own arrays) can detect up to 33 CYP2D6 alleles (variant forms of the gene), including gene duplications and deletions and three CYP2C19 alleles. An individual may have the CYP2D6*4 (rs3892097) and CYP2C19*2 (rs4244285) variants, which would interact with the probes on the microarray. When scanned, the label would be identified on the microarray at the position where the CYP2D6*4 and CYP2C19*2 probes are located. Microarrays and other technologies are helping bring efficient pharmacogenomic testing to all patient care settings where testing will become a standard of care.

REPORTING RESULTS

The results of pharmacogenomic testing are typically provided using the “star (*)” designation for an individual’s genotype. For instance, Figure 4a presents the results for CYP2C19 testing as presented by a large national laboratory (here based on a blood sample). In this case, the patient was homozygous for the *17 allele (Table 2). While pharmacists must understand this presentation of results, it is likely that reporting of results will need to be presented by metabolizer phenotype. In the example in Figure 4a, the patient’s metabolic phenotype is ultrarapid metabolizer (UM). A consistent reporting nomenclature must be implemented so all health care providers have a clear understanding of the results.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has developed a number of pharmacogenomic-based dosing guidelines. The guidelines refer to results being reported by allele and metabolic phenotype. For instance, related to Figure 4a, dosing guidelines for clopidogrel are based on the “*” nomenclature which defines the patient’s metabolic phenotype (Figure 4b). Clopidogrel is a prodrug; patients who are intermediate or poor metabolizers may not get the full benefit of this drug.

When considering the pharmacogenomic test results for the patient as shown in Figure 4a, the algorithm in Figure 4b guides recommendations based on the “*” allele nomenclature related to the metabolic phenotype. Here the *17 (CYP2C19 allele 1)/*17 (CYP2C19 allele 2)
results relate to a UM phenotype (Figure 4b). Reprinted by permission from Macmillan Publishers Ltd: [Clinical Pharmacology and Therapeutics] Scott SA, Sangkuhl K, Gardner EE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther. 90(2):328-332, copyright 2011. Importantly, the patient needs to understand the results are specific for a gene that codes for a drug metabolizing enzyme. The results should not be discussed relative to any other potential, such as relative risk of disease. This can help alleviate stress related to genetic information. For instance, an individual may not want to know their relative risk of Alzheimer’s disease based on their genetics. Explaining that the pharmacogenomic test is only looking at drug metabolism for instance will remove the patient’s anxiety related to learning about relative risk of having a potential devastating disease. This helps the patient realize that their genetic data is being used appropriately.

HOW TO INCORPORATE INTO COMMUNITY PHARMACY PRACTICE?
One of the main questions to understand in the application of PGx into community pharmacy practice is to move from scientific knowledge to understanding the clinical impact of the science. Currently, genomic information is known for many medications. However, as seen in Table 5 below, currently guidelines have only been established for a few classes of medications. Even within these classes, the question can still remain how to apply the information to improve patient care.

---

**Figure 4a. CYP2C19 Testing Results for an Ultrarapid Metabolizer (UM) as Presented by a Large National Laboratory**

**Indication for testing: Assess genetic risk for impaired CYP2C19-mediated drug metabolism**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 Specimen</td>
<td>Whole Blood</td>
</tr>
<tr>
<td>CYP2C19 Allele 1</td>
<td>*17</td>
</tr>
<tr>
<td>CYP2C19 Allele 2</td>
<td>*17</td>
</tr>
<tr>
<td>CYP2C19 Gene Mutation Interpretation</td>
<td></td>
</tr>
</tbody>
</table>

Result: TWO INCREASED-FUNCTION CYP2C19 ALLELES DETECTED

Recommendations: Consultation with a clinical pharmacy professional to discuss drug and dose selection is recommended. Detection of allelic variants does not replace the need for therapeutic drug and clinical monitoring as pharmacokinetics and drug response may be affected by other genetic and non-genetic factors.

---

**Figure 4b. Algorithm Related to the Clopidogrel-CYP2C19 Interaction**

ACS/PCI patient population

Initiate antiplatelet therapy with standard dosing of clopidogrel

CYP2C19 testing if genotype is unknown

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM</td>
<td>Standard dosing of clopidogrel</td>
</tr>
<tr>
<td>EM</td>
<td>Standard dosing of clopidogrel</td>
</tr>
<tr>
<td>IM</td>
<td>Prasugrel&lt;sup&gt;a&lt;/sup&gt; of other alternative therapy</td>
</tr>
<tr>
<td>PM</td>
<td>Prasugrel&lt;sup&gt;a&lt;/sup&gt; of other alternative therapy</td>
</tr>
</tbody>
</table>

<sup>a</sup> Depending on prescriber preference and patient characteristics.
For instance, guidelines are established for warfarin and genetic dosing information exists in the FDA approved drug labeling for this drug. On the side against using genetic testing with warfarin, labs are typically performed at least weekly when starting the medication to adjust the dose, so any needed adjustments can be made based on the lab findings and will be caught quickly. With the variability caused by factors like eating habits and the influence other medications being used at the same time, genetic testing may be an unnecessary additional cost. However, at the same time and on the other side of the argument, according to the CPIC guidelines, dosing may be useful in up to 40 percent of patients, those requiring less than 21 mg/week or more than 49 mg/week. Given the potential consequences of adverse events from warfarin use and the significance of the ischemic events warfarin is used to prevent (such as myocardial infarction, thromboembolic events), genetic testing may be warranted to enable clinicians to get the desired therapeutic level as quickly as possible while maintaining the safety of the patient. However, guidelines published in the journal Chest currently recommend against routine genetic testing prior to initiation of vitamin K antagonist therapy. Generally, genetic testing specific to warfarin is not being seen in practice but is receiving a lot of attention to understand how to best apply the technology.

Perhaps the most widely cited use for genetic testing with medications is with Plavix® (clopidogrel). The drug labeling for clopidogrel has a black-box warning recommending genetic testing be considered. The reason for this is that 12-15 percent of people cannot metabolize clopidogrel from its prodrug state to its active form, with some estimates stating this to be as high as 30 percent. The specific SNP variation involves the CYP2C19 pathway where people with the *1/*2 or *1/*3 alleles are intermediate metabolizers and *2/*2, *2/*3, *3/*3 are considered poor metabolizers (as seen in Figure 4b). According to CPIC guidelines, IM and PM individuals should receive an antiplatelet drug other than clopidogrel (such as prasugrel or ticagrelor).

Warfarin and clopidogrel provide examples of a controversial and a more generally accepted use of pharmacogenomic testing, respectively. To be able to apply this emerging knowledge clinically, health care providers will need to learn and rely on treatment guidelines being established. Pharmacists will need to be able to communicate this application both to prescribers and to patients. As mentioned earlier, one source pharmacists can turn to are CPIC guidelines. Currently, CPIC has published pharmacogenomic-based dosing guidelines for nine gene-drug pairs (Table 5). Other guidelines have either been submitted for publication or are in development. These guidelines are focused on how to apply test results. They do not dis-

<table>
<thead>
<tr>
<th>Gene/Drug</th>
<th>Status*</th>
<th>Gene/Drug</th>
<th>Status*</th>
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<td>HLA-B/allopurinol</td>
<td>10/2012</td>
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<td>12/2011</td>
<td>HLA-B/carbamazepine</td>
<td>5/2013</td>
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<td>DPD/5-FU,</td>
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*online publication - month/year, D = in development, S = submitted for publication
cuss whether or not a test should be ordered; instead they are based on having preemptive pharmacogenomic data, such as results available at the time of decision making.

More guidelines will be developed as data supports the use of pharmacogenomics to guide the dosing. The guidelines are available at http://www.pharmgkb.org/page/cpic and in the journal “Clinical Pharmacology and Therapeutics” as free access papers.

PAYMENT AND BILLING CONSIDERATIONS
Genetic testing and pharmacogenomics interpretation has the potential to get fully incorporated into pharmacy practice similar to immunizations. Widespread uptake will likely require a tested business model with reliable reimbursement to pharmacies; as was done with immunizations. While it can be more cost effective to conduct an entire panel of tests at one time, payment by third party payers may dictate if these tests are done together (bundled) or if single, specific genes are tested to assess interactions with the medications the patient is currently taking. It’s worth pointing out that physicians and medical practices are not allowed to receive payment by a lab (or other provider) for referrals. This is based on the Stark Anti-Kickback Regulations passed in 1993 that went into effect in 1995. This law would apply to pharmacists in this situation. Pharmacists and other health care providers should consult a health care attorney before agreeing to any financial arrangements when becoming or accepting a source of referrals.

The pharmacy business model may call for requesting reimbursement from the patient, billing the patient’s health plan (persistence may pay off) or billing the physician (under agreement if counseling is provided incident to an office visit) for consulting with patients or working with prescribers to change the patient’s medications. Provider status for pharmacists would simplify and clarify pharmacy billing for these services to insurance companies but is not an unconquerable barrier. Each of these scenarios needs to be vetted as the services are being established. Ultimately, trailblazers will determine how to make it feasible for patients to receive this much-needed service.

SUMMARY
Applying pharmacogenomics in pharmacy practice is an emerging service that can contribute significantly to improving patient outcomes with drug therapy and minimizing adverse events. The service delivery of collecting the DNA sample and communicating the results fits easily into the workflow of a pharmacy and provides pharmacists a further opportunity to extend their practices as the medication experts. Compensation mechanisms need to be determined for this emerging service. While this is being done, pharmacists should deepen their understanding of this area and identify how they can further help patients by incorporating this knowledge into their practice.

CASES
Case 1
Beza S is a 5-year-old Ethiopian girl who moved to the United States with her parents and two siblings three years ago. Beza was experiencing recurrent tonsil infections and her parents noticed she was snoring and “breathing funny.” Upon physical examination and with the history presented by her parents, Beza was diagnosed with obstructive sleep apnea syndrome (OSAS). Adenoid and tonsil hypertrophy (adenotonsillar hypertrophy; AT) is the most frequent cause of OSAS. The principal treatment is adenotonsillectomy, which was the course of action in caring for Beza. The procedure was performed and there were no procedural complications. Post-procedure analgesia treatment included one teaspoonful of acetaminophen 120 mg/codeine phosphate 12 mg syrup every six hours. The following day Beza was found to be “extremely sedate” and could “hardly open her eyes.” Her parents brought Beza to the emergency department (ED) at the regional hospital where the sedation, lethargy and a decreased respiratory rate were noted by the ED staff. A toxicology screen identified opioid toxicity due to morphine. With codeine being the suspected source of morphine, it was discontinued. Beza received supportive care and fully recovered. Upon discussion with the pharmacist, the parents consented to have Beza’s DNA tested to identify her cytochrome P450 2D6 (CYP2D6) genetics and metabolic phenotype. Ge-
netic testing results showed Beza had multiple copies of the normal active CYP2D6 gene, making her an ultrarapid metabolizer (UM), here converting excessive amounts of codeine to morphine.

There are many variants of the CYP2D6 gene which can result in a range of metabolic phenotypes including poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and UM's. The frequency of certain variants seen in a given ethnic group can differ greatly. The prevalence of UM's in Caucasians is approximately 3-7 percent, while it approaches 30 percent in African Ethiopians. The case of Beza is similar to other reports, including those reporting fatalities in children due to morphine toxicity following codeine administration. These cases served as the basis for the FDA decision to issue a “black box warning” relative to contraindicating codeine use in children for analgesia following surgery for AT. The use of codeine in various metabolic phenotypes has been presented by Crews et al.

Case 2
Thomas H is a 57-year-old Caucasian male with a history of depression. He is currently receiving citalopram 30 mg/day having not responded to the initial 20 mg/day dose. Thomas has recently been complaining of “heartburn” and with him experiencing this multiple times per week he was started on omeprazole 20 mg/day and has now been taking the drug for three weeks. Thomas now arrives at his physician’s office for a comprehensive annual check-up. Thomas surprises his physician by presenting her with genetic testing information provided by a direct-to-consumer genetic testing company. While the physician appreciates Thomas’ interest in his genetics, she is not entirely sure what to do with the information. Thomas’ physician speaks with the local pharmacist as much of the genetic information provided to her was related to “drug response.” The pharmacist responds that except for the drug metabolizing enzyme CYP2C19, Thomas appears to be a “normal” metabolizer (see Table 1). The genetic information related to the CYP2C19 gene indicates that Thomas is considered an “intermediate metabolizer” having lower metabolism than a “normal” metabolizer. Here, with one normal function gene (CYP2C19*1) from one parent and one loss-of-function gene (CYP2C19*2) from Thomas’ other parent. The annual check-up shows that Thomas has an abnormal electrocardiogram with a prolonged Q-T interval, putting him at risk for torsades de pointes, a serious, life-threatening arrhythmia. The physician reviews the information and questions the pharmacist about using citalopram for Thomas. She explains that the literature indicates CYP2C19 poor metabolizers taking citalopram are at risk of a prolonged Q-T interval, but notes Thomas is an intermediate metabolizer. The pharmacist recognizes that omeprazole is an inhibitor of the CYP2C19 enzyme and that in Thomas’ case is causing him to be a PM. As a “genetic IM,” omeprazole further decreases enzyme function essentially turning Thomas into a PM. The pharmacist suggests an alternative to omeprazole with less CYP2C19 inhibition. A follow-up electrocardiogram shows a normal Q-T interval and Thomas is maintained on citalopram, to which he is responding.

The interaction of a drug with a gene variant, such as CYP2C19*2, a loss-of-function form is the defined drug-gene interaction seen in Thomas. The addition of omeprazole results in a drug-drug-gene interaction resulting in phenocconversion, where Thomas is converted from an intermediate metabolizer to a poor metabolizer. Here, the pharmacist has integrated his/her drug interaction expertise with pharmacogenomics to aid in optimal patient care. 

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Implementing Pharmacogenomics in Pharmacy Practice
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CONTINUING EDUCATION QUIZ
Select the correct answer.

1. The human genome project:
   a. Sequenced all 30,000 to 50,000 genes
   b. Paved the way for genetic testing
   c. Took two extra years to complete
   d. Both a and b

2. Which if the following would be considered a SNP?
   a. Guanine replacing thymine in a DNA sequence
   b. Threonine replacing cysteine in a DNA sequence
   c. PM
   d. Both a and b are correct

3. Which of the following proteins are NOT important in pharmacogenomics?
   a. Transporters
   b. Receptors
   c. Metabolizing enzymes
   d. All of the above are important.

4. Consider a patient taking an active drug. Which metabolizer phenotype would likely result in the smallest AUC on a concentration versus time graph?
   a. PM
   b. EM
   c. UM
   d. IM

5. You have a patient that is a CYP2C19 PM and is taking a standard dose of an active drug primarily metabolized by CYP2C19. What could be a potential concern in this patient?
   a. Inefficacy
   b. Nothing. If the body can’t use a CYP2C19 enzyme the body will adjust and simply use a different enzyme.
   c. Nothing. Since the patient has a “normal” metabolism a standard dose should work.
   d. Toxicity

6. A genetic variation resulting in decreased activity of an influx transporter in hepatic tissue would result in which of the following?
   a. A larger volume of distribution
   b. A higher concentration of drug in the blood, but with the same pharmacokinetic parameters.
   c. A smaller volume of distribution
   d. Lower concentrations of drug in the blood
7. Polymerase Chain Reaction (PCR):
   a. Amplifies a single gene or a portion of a gene
   b. Can only use two primers, so it can only check for one form a gene
   c. Amplifies the whole chromosome
   d. Is done with an electric current passed through a gel

8. Gel electrophoresis:
   a. Uses magnetic forces to separate DNA segments based on size
   b. Uses an electric current to separate DNA fragments based on size
   c. Amplifies DNA
   d. Uses the positive charge on DNA to separate DNA fragments based on size

9. Genetic variations:
   a. Can be explained only by SNPs
   b. Always have a clinical implication
   c. Can influence drug response
   d. Are very rare

10. A patient, started on a low dose of warfarin, comes into your pharmacy complaining that he is “bruising” very easily and has an “almost constant” bloody nose. One possible explanation for this is:
   a. The patient has a genetic variation that decreased the production of VKORC1, making him more sensitive to warfarin.
   b. The patient has a genetic variation that decreased the production of VKORC1, making him metabolize warfarin faster and leading to toxicity.
   c. The patient has a genetic variation that increased the production of VKORC1, making him more sensitive to warfarin.
   d. The patient has a genetic variation that increased the production of VKORC1, making him metabolize warfarin faster and leading to toxicity.

11. A patient with two *2 alleles for CYP2C19 would be considered what?
   a. Homozygous and a CYP2C19 IM
   b. Heterozygous and a CYP2C19 IM
   c. Heterozygous and a CYP2C19 PM
   d. Homozygous and a CYP2C19 PM

12. Consider a prodrug such as codeine. Which of the following phenotypes would likely have the greatest AUC for the parent drug concentration?
   a. UM
   b. IM
   c. EM
   d. PM

13. What therapeutic action would be most appropriate when considering an EM using a prodrug?
   a. Since the patient is an extensive metabolizer, he would metabolize the drug faster and would need a lower dose.
   b. Since the patient is an extensive metabolizer, he would metabolize the drug faster and would need a higher dose.
   c. The patient likely metabolizes the drug at the expected rate. Neither the dose nor the drug need to be changed based on phenotype.
   d. Since the patient is an extensive metabolizer, he would metabolize the drug faster and would be at an increased risk of toxicity and would receive an alternative drug.

14. An increased function of efflux transporters in the gastrointestinal track would potentially result in:
   a. No change. The body will stop transporting once equilibrium is reached.
   b. An increased bioavailability
   c. A decreased bioavailability
   d. Decreased clearance
15. In order for PGx to be incorporated into pharmacies what must happen?
   a. Patients need to routinely request pharmacogenomic testing.
   b. Pharmacists need to learn what tests are available and how to apply the results when selecting or modifying drug therapy.
   c. Physicians need to start demanding pharmacogenomic testing.
   d. Laws must be passed that require pharmacogenomic testing.

16. What are efficient ways to perform PGx testing in pharmacy?
   a. Buccal cheek swabs or saliva samples
   b. Blood or urine tests
   c. Urine or saliva samples
   d. Any biological substance can be used, because all cells have DNA

17. Which of the following is true for a CYP2C19 UM patient on clopidogrel?
   a. Although the patient has a faster metabolizing rate of the drug, a standard dose of clopidogrel can be used.
   b. The patient has a faster metabolizing rate of the drug, so a lower dose is needed.
   c. The patient has a faster metabolizing rate of the drug, and the safest option is to switch the patient to a drug that is not metabolized by CYP2C19.
   d. CYP2C19 UM are so rare that there are no recommendations for therapeutic actions.

18. What is an allele?
   a. A 20 to 30bp synthesized sequence to compliment a gene of interest.
   b. A single change in a nucleotide
   c. One of two or more alternative forms of a gene
   d. One possible form a gene

19. Pharmacists must be concerned with which of the following regulations when getting paid for delivering lab tests?
   a. The Medicare Modernization Act of 2003
   b. The Stark Anti-kickback regulations from 1993
   c. CLIA lab certification
   d. The 1962 Kefauver-Harris Amendment

20. While many drug labels have genetic information, the true test of the usefulness of this science in medical practice will be determined by:
   a. The ability to identify a patient’s ancestry through their DNA
   b. The statistical significance of the science
   c. The clinical utility of the knowledge
   d. The ability to identify a patient’s risk for diseases in the future