



Pharmacogenetics

Treating Disease Using an Understanding of Genetics

Case Study: Leukemia and the Thiopurine
Methyltransferase (TPMT) Enzyme

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November 2007

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Summary

The discipline of pharmacogenetics examines how genetic variations in an individual correlate with their responses to a specific medication. The ultimate goal of pharmacogenetics is to develop medical treatments tailored to the individual (1).

This lesson uses a ‘case study’ approach to investigate the applications of genetics to medicine. It explores one of the first examples of a pharmacogenetic test to enter mainstream clinical practice.

Through a brief fictional scenario, students are introduced to the disease involved (acute lymphocytic leukemia) as well as the wide range of individual response to the drug used to treat it. Then, students interpret data similar to those initially published in scientific journals in order to construct an understanding of how genetic variation can be used to ‘tailor’ medical care. Lastly, students are asked to apply their understanding of the lesson by making the appropriate medical recommendation based on a particular individual’s genotype.

Objectives

- Students will be able to use scientific/case study data and interpret graphs in order to draw conclusions.
- Students will understand how individual genetic variation can impact medical practice and clinical outcomes, using the example of TPMT.
- Students will be able to predict how polymorphisms in the gene for TPMT indicate courses of medical care for particular individuals.

Enduring Understandings

- Understanding the genetic profile of individuals has the potential for allowing customization in the way patient risks are assessed, and how medicines are designed and delivered.
- Genetic information and environmental factors interact to produce disease states, influence treatments, and impact medical outcomes.
- The incorporation of new technologies to medicine raises ethical issues, such as those related to resource allocation, drug testing, and genetic privacy. (*Extension*)

National Science Standards: Grades 9-12

<i>Science as Inquiry</i>	
Abilities necessary to do scientific inquiry	•
Understandings about scientific inquiry	•
<i>Physical Science</i>	
Structure and properties of matter	
Chemical Reactions	•
<i>Life Science</i>	
The cell	
Molecular basis of heredity	•
Biological Evolution	•
Interdependence of organisms	
Matter, energy, and organizations in living systems	•
Behavior of organisms	
<i>Science and Technology</i>	
Abilities of technological design	•
Understandings about science and technology	•
<i>Science in Personal and Social Perspectives</i>	
Personal health and community health	•
Science and technology in local, national, and global challenges	•
<i>History and Nature of Science</i>	
Science as human endeavor	•
Nature of scientific knowledg	•
Historical Perspectives	•

WA State Standards

GLE 1.2.6

Understand cellular structures, their functions, and how specific genes regulate these functions

GLE 1.2.7

Understand how genetic information (DNA) is encoded at the molecular level and provides genetic continuity between generations.

GLE 3.1.1

Analyze local, regional, national, or global problems or challenges in which scientific design can be or has been used to design a solution.

GLE 3.1.3

Evaluate consequences, constraints, and applications of solutions to a problem or a challenge.

GLE 3.2.1

Analyze how scientific knowledge and technological advances discovered and developed by individuals and communities in all cultures of the world contribute to changes in societies.

GLE 3.2.2

Analyze how the scientific enterprise and technological advances influence and are influenced by human activity.

Audience Targeted towards 10-12 Biology, Chemistry

Time Required Minimum one 50 minute period, and an additional day for debriefing would be helpful

Helpful

Prior Knowledge Mendelian Genetics, DNA structure and function

Pharmacogenetics - Treating Disease Using an Understanding of Genetics

Leukemia Case Study

(This is a fictitious scenario, but it is based upon clinical observations)

It's called the children's ward. For two teenagers who have been recently diagnosed with leukemia, it seems insulting to have their lives hijacked by doctors and nurses with stuffed animals clipped to their stethoscopes. Laura is a forward on her school soccer team and leads the league in scoring. For the last four months she has been really tired, but nothing seemed really wrong until her legs became covered with bruises. Just pressing her fingers on her skin was practically enough to make a bruise. It didn't seem real when her doctor, Jane Ryder, diagnosed her with Acute Lymphocytic (or Lymphoblastic) Leukemia (ALL), or when she told her that ALL is the most common malignant (spreading) cancer found in children. She's 14 years old; she's not a child!

Beth is 13 and looks remarkably like Laura. Both have straight dark hair, large brown eyes, and tall slender builds. Beth has never been that athletic; she prefers reading and theater. She's hoping to be part of the drama team next year when she goes to high school, even though she'll only be a freshman. But she's been missing a lot of school because of one virus after another, lots of fevers and night sweats, then that rash in the fall. Now she's in a hospital, and it seems like the only people she sees are her parents, Dr. Ryder, and the nurses.

Laura and Beth both have ALL, which arises from the uncontrolled growth of immature white blood cell (lymphocytes). These cells, which are 'stuck' in an early stage of development, become so numerous that they crowd out normal blood cells. Each year about 30 cases occur per million people, and most of those cases are in children aged 2-5 years. The cause of ALL remains largely unknown, although a small number of cases are associated with inherited genetic syndromes (2).

Laura and Beth are both suffering from anemia (low blood cell levels), fevers, bleeding, and are pale and thin. Dr. Ryder has decided to treat them as in-patients, keeping them in the hospital while treating them with a **'thiopurine' drug called 6-Mercaptopurine (6-MP)** known to be highly effective in treating leukemia. Thiopurines are very similar to the regular purine nitrogen bases, such as adenine and guanine, which make up DNA and RNA. The only difference is that thiopurines have an extra sulfur group attached to them. They are similar enough to a regular purine base that our cells convert them to nucleotides (with the addition of a deoxyribose sugar and phosphate). These modified **thioguanine nucleotides (TGN)** – are then incorporated into DNA.

The TGN nucleotides interfere with DNA replication and stop rapidly growing cells like cancer cells from further growth. Unfortunately, they also block the growth of other fast growing cells needed for good health, like the cells in the bone marrow that develop into red and white blood cells. As with many drugs given as chemotherapy, it is important to give a high enough dosage to prevent cancer cells from replicating, while avoiding damage to the normal

tissues. Too high a drug dose can be very toxic. Dr. Ryder gives both girls the *same* dosage of the drug before leaving the hospital for the night.

While making her rounds over the next few days, Dr. Ryder sees Laura's vital signs plummet. Her anemia has worsened; her red blood cell count is so low that her heart function could be compromised. Her fevers are spiking, and her breathing is becoming shallow and labored. She is not eating and is being hydrated intravenously. Her condition is life-threatening. In contrast, Beth's anemia has decreased, she is free of fever, and is actually showing signs of an appetite and boredom, good indicators of improved health. Dr. Ryder had not anticipated that the drug could act so differently in two individuals. Even as she looks at Beth's chart, she can picture Laura's body's struggle to hold its own just two private rooms away. Dr. Ryder knows she must find out why her patients are responding so differently. But where should she start, and will she find an answer in time to help Laura?

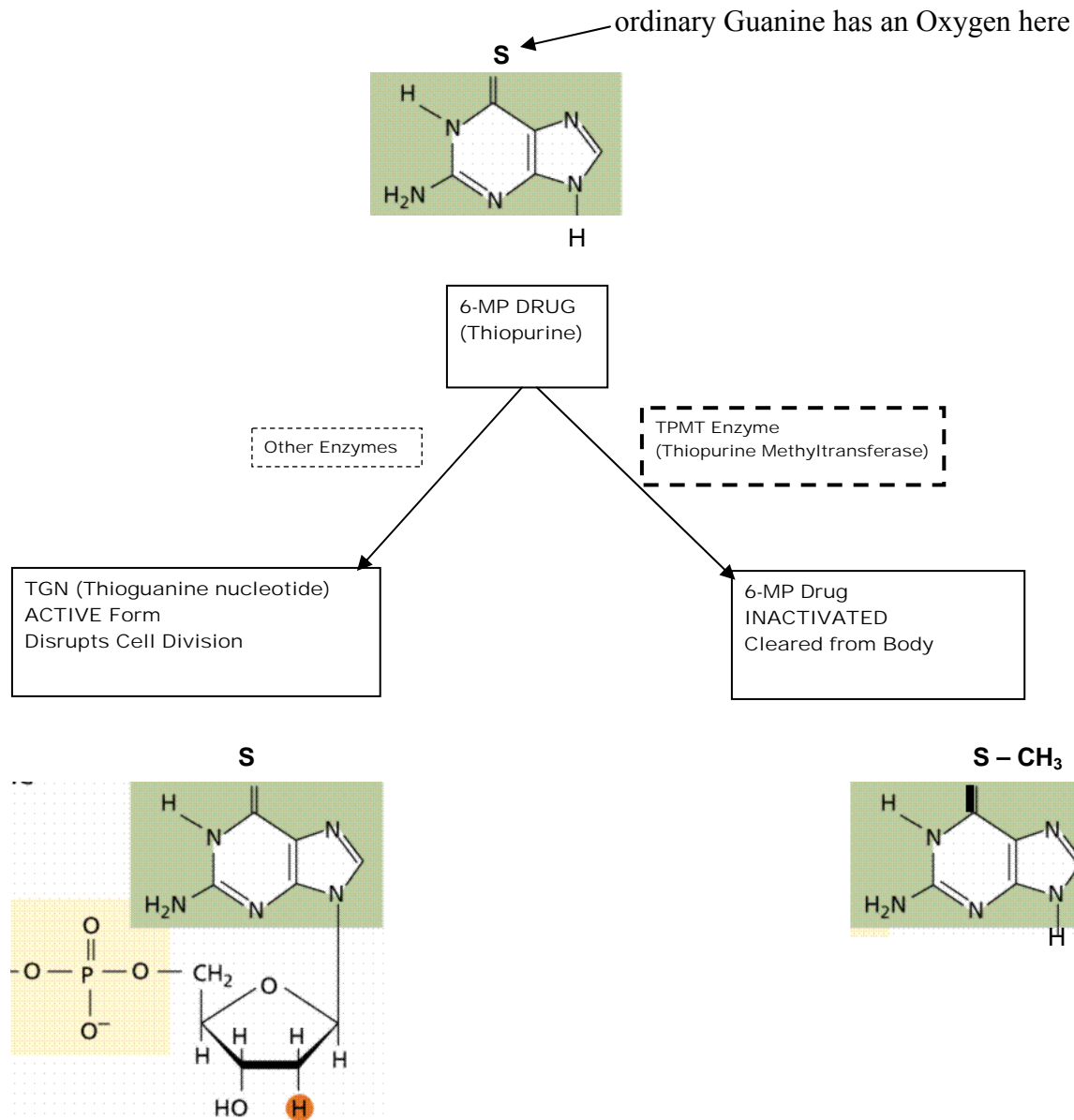
STEP 1: BRAINSTORMING

Suggest a reason why the drug might affect the two girls differently.

What types of things might Dr. Ryder test in order to determine how the two girls are reacting to the drug? Provide two or three ideas of possible things to test.

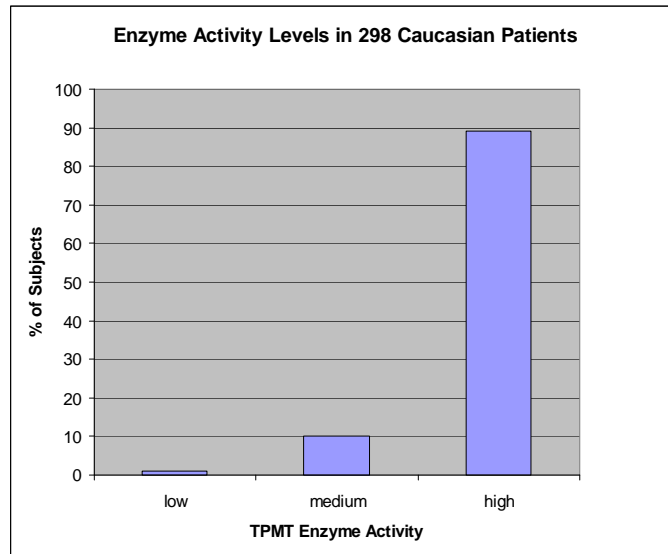
STEP 2: ENZYME ACTIVITY

Dr. Ryder learns that the difference in patient reaction to the drug probably has something to do with how the drug is naturally metabolized (processed) in the body to be removed. After searching the scientific literature, she learns that the **drug 6-MP** can either be converted to the active form, **TGN nucleotides**, or can be inactivated **with the help of the TPMT enzyme (Thiopurine methyltransferase)**. *Within each patient that takes the drug, both processes are occurring and they compete with each other.*



Since the therapy aims to harm quickly replicating cells, without overly impacting normal ones, it is important that excess drug is inactivated. She decides to see how levels of the TPMT enzyme activity might vary between people.

She reviews the research papers that have been published about the TPMT enzyme and finds an interesting graph. From a study of 298 randomly selected Caucasian individuals, researchers found the following levels of TPMT enzyme activity:



2a. If Dr. Ryder had 10 Caucasian patients in the next month, how many would you *predict* to have each of the TPMT Enzyme Activity Levels, based on the graph above?

Low-

Medium-

High-

Would you expect the *actual* values to be different? Why might there be differences?

2b. Each individual inherits two copies of the gene for the enzyme, one from each parent. Dr. Ryder suspects that variation in enzyme activity level is controlled by two different versions (alleles) of that gene. Does this graph (and the number of phenotypes) suggest that enzyme activity levels are based on a dominant/recessive or a codominant pattern of inheritance? Explain your answer.

2c. Which bar (low, medium, or high) represents individuals who might be *homozygous for a 'low enzyme activity' version of the gene*? _____ Which bar represents individuals who might be *homozygous for a 'high enzyme activity' version of the gene*? _____ Which bar represents *heterozygotes*? _____

2d. Answer the question, “How does enzyme activity level vary among the patients examined?” In your answer, be sure to include supporting data from the graph above. Explain how these data support your conclusion.

2e. Challenge question: The actual graph (below) showed much more detail. Why do you think that there is more variation between patients than shown in the simplified graph?

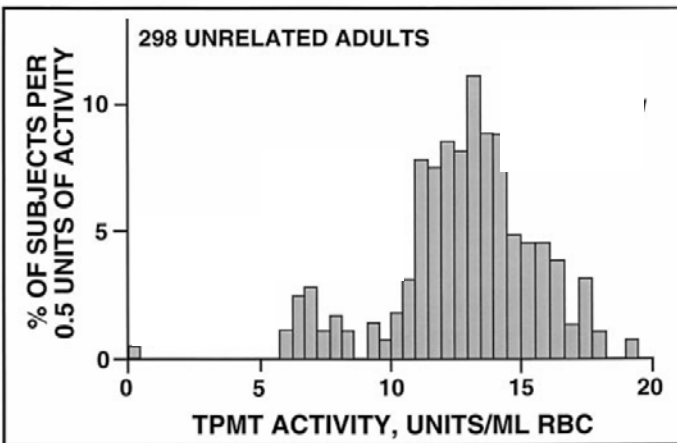


FIG. 1. RBC TPMT frequency distribution histogram for 298 randomly selected Caucasian subjects.

From Weinshilboum and Sladek (1980). (3)

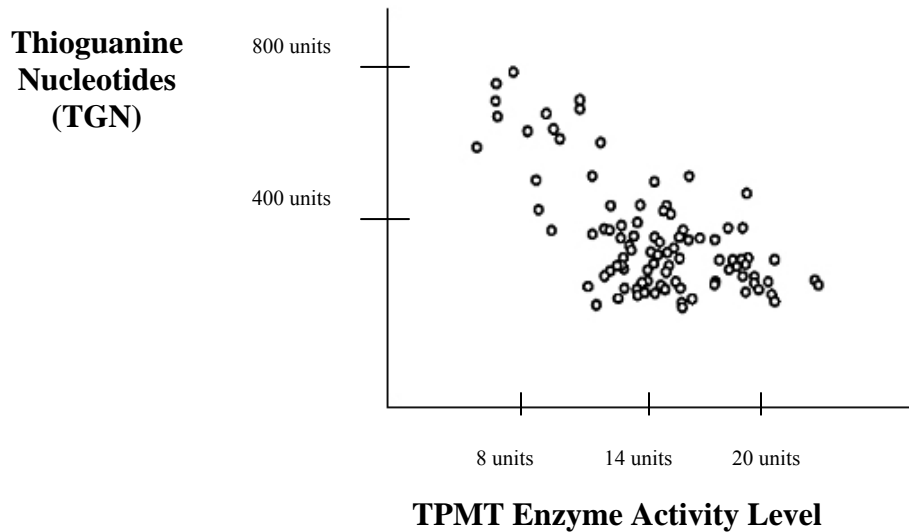
STEP 3: TESTING THE PATIENTS FOR TPMT ENZYME ACTIVITY LEVELS

Dr. Ryder then tested Laura, who was very sick, and found that her TPMT enzyme activity level was extremely low.

3a. Why would individuals with the lowest level of enzyme get the sickest when they take the drug? Suggest one possible reason:

Investigating further, Dr. Ryder decides to look at drug levels in many patients who are all receiving the *same standard* doses of the thiopurine drug and compare them to enzyme levels. When she compares the level of Thioguanine Nucleotides (TGN) created by the thiopurine drug to the body's level of TPMT enzyme in patients, this is what she finds.

Thioguanine Nucleotide Concentrations and TPMT Enzyme Activity Levels
(in 95 Children with Acute Lymphoblastic Leukemia (ALL) who were being treated with standard doses of Thiopurine Drugs)



(modified from Lennard, 1990, and reprinted with permission)(4)

3b. Describe the relationship between TPMT enzyme activity levels and TGN levels. Be sure to include supporting data from the graph.

STEP 4: PUTTING IT ALL TOGETHER

4a. Circle the appropriate response (either high or low, heterozygous, homozygous) in the following sentences.

From her research, Dr. Ryder hypothesized that patients such as Laura (who became very sick upon receiving the drug) have very **high / low** TPMT enzyme activity and therefore very **high / low** levels of TGN nucleotides at normal doses. *They easily became sick from the effects of the drug*, and could even die. These patients are **homozygous / heterozygous** for the version of the gene encoding **high / low** enzyme activity. A better drug dose for these patients is 1/10th the level of other patients.

Most patients need a normal (or sometimes even larger-than-normal dosage) for the treatment to be most effective. Such patients are most likely **homozygous / heterozygous** for the version of the gene encoding **high / low** TPMT enzyme activity.

Based on the graph in Section 2, about 10% of the Caucasian population is **homozygous / heterozygous** for the version of the gene encoding **high / low** enzyme activity.

STEP 5: SNPs and TPMT

New DNA techniques reveal TPMT gene is located on chromosome 6, is about 34 kilobases in length (34,000 DNA bases), and has 8 ‘exons’. An exon is a region of a gene that is present in the final functional transcript (mRNA) from that gene. The diagram below shows a representation of the TPMT gene, showing the exons as boxes. The first ‘wild type’ is the most common version. In our case, the second **version of the TPMT gene is associated with low enzyme activity (TPMT*3A)** and has two single nucleotide polymorphisms (SNPs), or changes in single DNA nucleotide bases (from ‘G’ to ‘A’ in one case and from ‘A’ to ‘G’ in another) that result in different amino acids being inserted in the enzyme. This, in turn, affects the enzyme’s function. Over 20 different gene variants have been found, three of which are shown below.

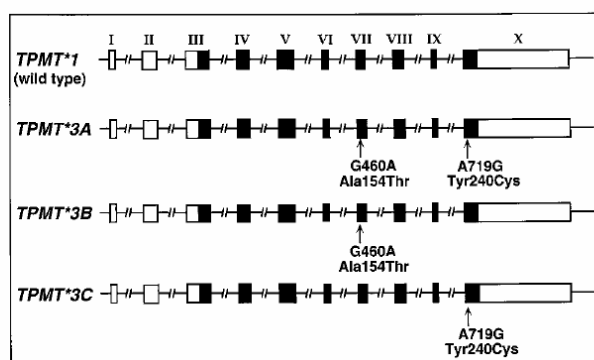
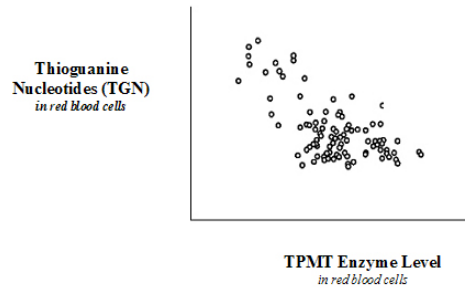


FIG. 4. Selected human TPMT alleles.

The wild-type human TPMT allele (*TPMT*1*) and variant alleles *TPMT*3A*, *TPMT*3B*, and *TPMT*3C*. Rectangles represent exons, with black coding areas and white untranslated regions.

Weinshilboum, 2001 (5)

5a. Dr. Ryder now has the ability to conduct a SNP genetic test on her patients to determine what level of drug they should get. A new patient on the ward, Kevin, is **homozygous for TPMT 3A***. The graph shown in STEP 3 is shown below. *Circle the area of the graph that might likely correspond to Kevin's TGN and Enzyme Activity Levels.* Explain why you circled that region.



5b. What level of the drug (low, medium, or high) should Dr. Ryder give him? Explain your answer.

5c. In your own words, summarize how knowing someone's TPMT DNA sequence could be used to determine what kind of medical care they should receive.

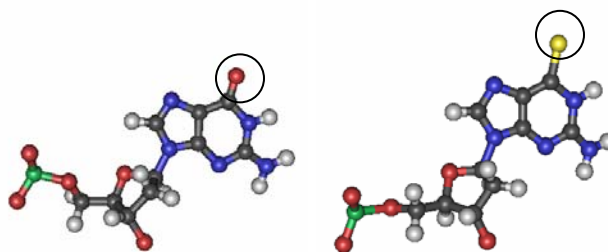
Molecular Modeling of Structures

Download the Cn3D software, if it is not already loaded on your computer.

Cn3D is available at NCBI-Structure: <http://pubmedexpress.nih.gov/Structure/CN3D/cn3d.shtml>

Guanine and Thioguanine

1. Go to <http://www.ncbi.nlm.nih.gov/Structure> and type 1N17 into the search box. Click on 1N17.
2. Click VIEW 3D STRUCTURE, and then OPEN.
3. When the structure opens, go to the STYLE menu and pick RENDERING SHORTCUTS → Ball and Stick.
4. In the Sequence/Alignment Viewer window *below* the picture, click on a 'g' (guanine) and watch it light up yellow in the structure.
5. Back in the structure picture window, go to the SHOW menu and pick SELECTED RESIDUES. Everything except guanine should go away.
6. Go to STYLE→COLORING SHORTCUTS→ELEMENT to show the different atoms. (you may need to click in the sequence/alignment viewer window to make the colors show again)



Guanine nucleotide
Oxygen circled

Thioguanine nucleotide
Sulphur circled

7. Go to SHOW-> Everything to see the whole DNA helix again.
8. Repeat steps 4-6 above, but this time highlight the letter 'n' in the Sequence/Alignment Viewer. That represents the Thioguanine. You should be able to see a yellow Sulphur atom attached where an Oxygen was in Guanine.
9. Go to SHOW-> Everything to see the whole DNA helix again.
10. Go to STYLE->Rendering Shortcuts->Space fill. Now you can really see the impact of that Sulphur atom!

Human thiopurine S-methyltransferase (TPMT Enzyme)

1. Go to <http://www.ncbi.nlm.nih.gov/Structure> and type 2BZG, into the search box
2. Click VIEW 3D STRUCTURE, and then OPEN. The parts represented in ball and stick form are the parts of the structure that vary depending on the allele.

Pharmacogenetics and its Social Context – For Teachers

The use of genetic information to determine how medicine is practiced involves many ethical, legal and social issues. For example:

Resource allocation issues

Developing treatments based on genetic information is costly and time-consuming. Is it ‘fair’ use of our resources as a society to focus on developing such technologies, especially when they may benefit a limited number of people, or be available only to those who could afford them? How is such research situated in the larger context of health care disparities in our country?

Genetic privacy issues

How can we be sure that genetic information is used to benefit patients and not discriminate against them? What are the implications of having genetic information about your health? Who should have access to that information? Should that information be available to prospective employers and insurance agencies? Should laws be enacted to protect genetic privacy? Is it fair to insurance agencies if you know you are at risk for a certain disorder and you don’t divulge that information?

In certain cases, such as the TPMT one described in this lesson, knowing one’s genetic status can lead to specific medical actions. Also, since TPMT is not currently known to cause disease, the genetic information is not stigmatizing.

Ethnic/Racial differences and allele types

Different alleles are often found to be associated more frequently with particular ethnic/racial groups. In the TPMT example, *3A allele is most frequently found in white European populations, is less frequently found in African Americans, and has never been seen in East Asians. The *3C allele is the most common variant in East Asian populations. How will the discovery of genetic information about populations and the utilization of that information for medical purposes impact our understanding of race?

TPMT and the FDA

In 2003, the FDA analyzed information available about the TPMT enzyme and its role in processing thiopurine drugs, in order to decide whether or not to require testing of patients prior to administration of those drugs. They decided against mandatory testing for several reasons:

- Testing would be costly, especially given that there are so few people who are strongly negatively affected by the drug (1 in 300 Caucasians).
- Patients are already watched closely when the drugs are administered.
- Doctors may shy away from using a potentially life-saving drug because of the added burden of testing.
- The information about whether heterozygotes (people in the intermediate range of enzyme activity, with one copy of the normal activity and one copy of the low activity alleles) benefited from testing was not conclusive.

Instead of requiring patient testing, the FDA required labeling of the drug packaging. While a few institutions test their patients, they are not required by law to do so.

In 2005, the FDA announced the first drug that would require testing before it could be prescribed (Aczone, an acne drug) (6).

Drug makers will be greatly influenced by the new developments in this field – they will be able to sell their drugs to potentially fewer people, but those people are more likely to benefit from the drugs and less likely to file damaging lawsuits.

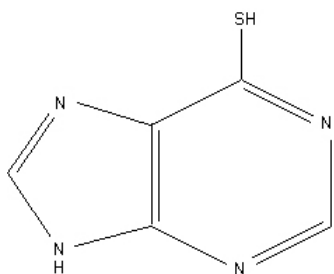
The fear of lawsuits may also drive doctors to recommend tests. Ethical dilemmas may arise for doctors whose patients want drugs that may not be recommended for them based on their genetic profile. There are private companies such as *GeneLex* that now offer tests privately to individuals who are interested in learning their genotypes in genes related to drug processing.

Potential Extension: Students could propose an FDA policy for TPMT as part of an ethics unit, then look at what the FDA developed.

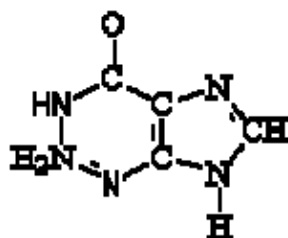
1. THE DRUG

Thiopurine Drugs: How they work

- Thiopurine drugs such as 6-mercaptopurine, 6-thioguanine, and azathioprine are used to treat Acute Lymphocytic Leukemia, autoimmune disorders, inflammatory bowel disease, and organ transplants.
- They are guanine analogs, and have a sulfhydryl group attached to C6 (see below).
- Thiopurines are 'prodrugs' and are inactive until they are processed by the body into active form
- Thiopurines are converted in the body to thioguanine nucleotides, which can be incorporated into DNA during replication.
- When incorporated into replicating DNA, the altered nucleotides stop the process of replication from continuing. Thiopurines can also impact the purine biosynthesis pathway.
Thiopurines especially affect cells that are frequently replicating (such as cancer cells).
- Thiopurines have a 'narrow therapeutic index', which means that they can be very toxic if not administered correctly. The ideal dosage is high enough to damage frequently dividing cancer cells but low enough that excess drug can be cleared with the aid of the TPMT enzyme.
- When the thiopurine drug levels are too high, a life threatening condition called myelosuppression can occur. This is a decrease in the production of white blood cells, red blood cells, and plasma cells in the bone marrow.



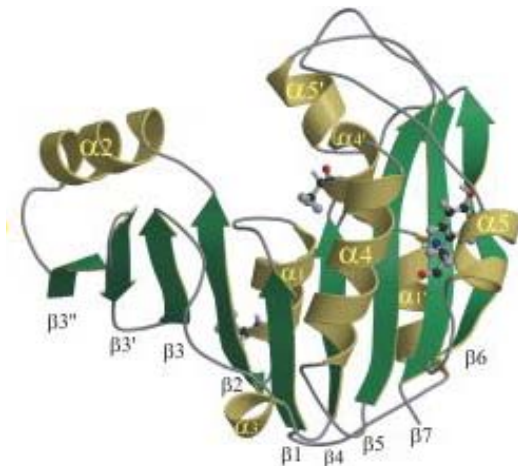
6-Mercaptopurine, a Thiopurine Drug
(*analog of the nucleotide precursor guanine*)



Guanine
2003 A.M. Helmsartina

2. THE ENZYME

How the Thiopurine Methyltransferase (TPMT) enzyme found in the body metabolizes the Thioguanine Nucleotides (TGN) created by the Thiopurine drug



Three-dimensional structure of TPMT (7)

Side chains are illustrated for the residues involved in the most common polymorphisms

- The TPMT enzyme adds a methyl group (CH_3) *on to* the sulfhydryl group (SH), which prevents the incorporation of the thioguanine drugs into replicating DNA.
- The enzyme helps the body metabolize/process the thiopurine drugs by limiting their potency ('deactivating').
- Several different genetic polymorphisms of the gene result in an enzyme that has low activity, probably because the enzyme is more susceptible to degradation.
- In a large population of Caucasians, 89% were homozygous for 'High Activity' alleles, 11% heterozygous (one 'High' and one 'Low', resulting in intermediate activity), and 1/300 were homozygous for Low Activity. (Later reports of East Asians found no such distribution among them). (5)
- Those individuals homozygous for Low Activity alleles are not able to process the thioguanine nucleotides well, and when the thiopurine drug is administered at the usual level it can result in death.
- The natural substrate for TPMT has not yet been identified.

3. THE GENE

Genetic variation in the TPMT gene

- The TPMT gene is 34kb in length and consists of 10 exons
- It is located on the short arm of chromosome 6
- While several variant alleles exist, TPMT*3A is the most common variant allele for low TPMT activity in Caucasians. It is currently thought that 6 variants exist (8).
- TPMT*3A has two single nucleotide polymorphisms (SNPs) that alter the encoded amino acids (i.e., they are nonsynonymous SNPs).
- In Asians, TPMT*3A is either not present or occurs at very low frequency, and TPMT*3C is the most common variant allele.
- In addition, The 5' region of the TPMT gene includes a variable number tandem repeat (VNTR) in which a 17 or 18 base pair element is repeated from four to eight times. Studies have suggested an inverse relation between the total number of VNTRs on both chromosomes and TPMT activity.
- Researchers at several institutions, including St. Jude's Children's Hospital in Memphis, TN and at the Mayo Clinic in Rochester, MN, screen patients to determine their TPMT genotype before administering chemotherapy with thiopurine drugs.

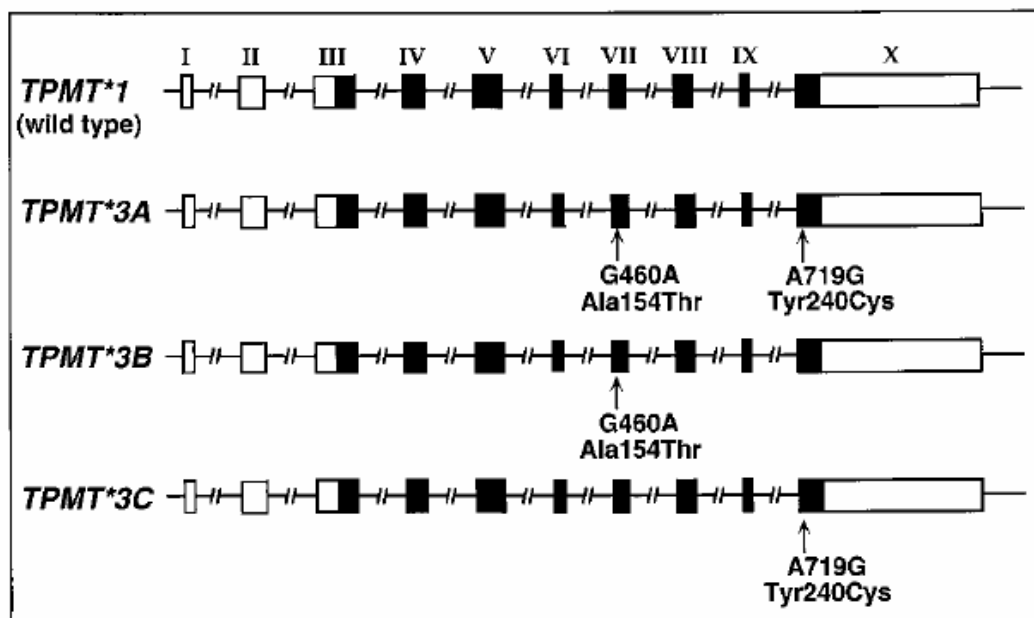


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Weinshilboum, 2001 (5)

Genetic Variation and Drug Responses

Background Information for Students

Differences in DNA - SNPs

Scientists have determined that over 99% of DNA is identical between people. That means that differences in the remaining 1% account for the wide range of variation we see in humans. This variation most commonly takes the form of Single Nucleotide Polymorphisms (poly=many, morph=form), or SNPs – single base changes in DNA (from A to C, for example) that occur approximately once every 300 bases. Since human DNA has about 3 billion base pairs, there are about 1 million SNPs. For a SNP to count as a polymorphism, it must be present in at least 1% of the population.

Why do SNPs matter? What impact do they have?

SNPs can serve as indicators – markers – associated with particular characteristics.

Occasionally, a change in a base occurring in a gene will change the protein product that gene codes for. For example, the genetic disease Sickle Cell Anemia is caused by a single base change in the DNA coding for hemoglobin, the protein that carries Oxygen in the blood.

However, SNPs do not usually cause disease, but rather can help indicate the chance that someone may develop one. For example, a gene associated with Alzheimers' disease (ApoE) is found in many variants. Inheriting one of the variants, E4, results in a change in the protein product that is associated with an increased risk of developing Alzheimer's (9).

SNPs may occur in a part of the DNA that is not transcribed into RNA and translated into protein (a 'noncoding' region). However, they may still impact the resulting protein if they impact how a gene is regulated or how a protein is spliced together. SNPs may also specify the same amino acid in the resulting protein as the original sequence did (a 'silent substitution').

A group of SNPs together on a chromosome is termed a 'haplotype'. Sometimes haplotypes can also be associated with risks for disease. Scientists are trying to map these 'haplotypes' to create a HapMap – a picture of the variation in the human genome.

Drug Reactions and SNPs

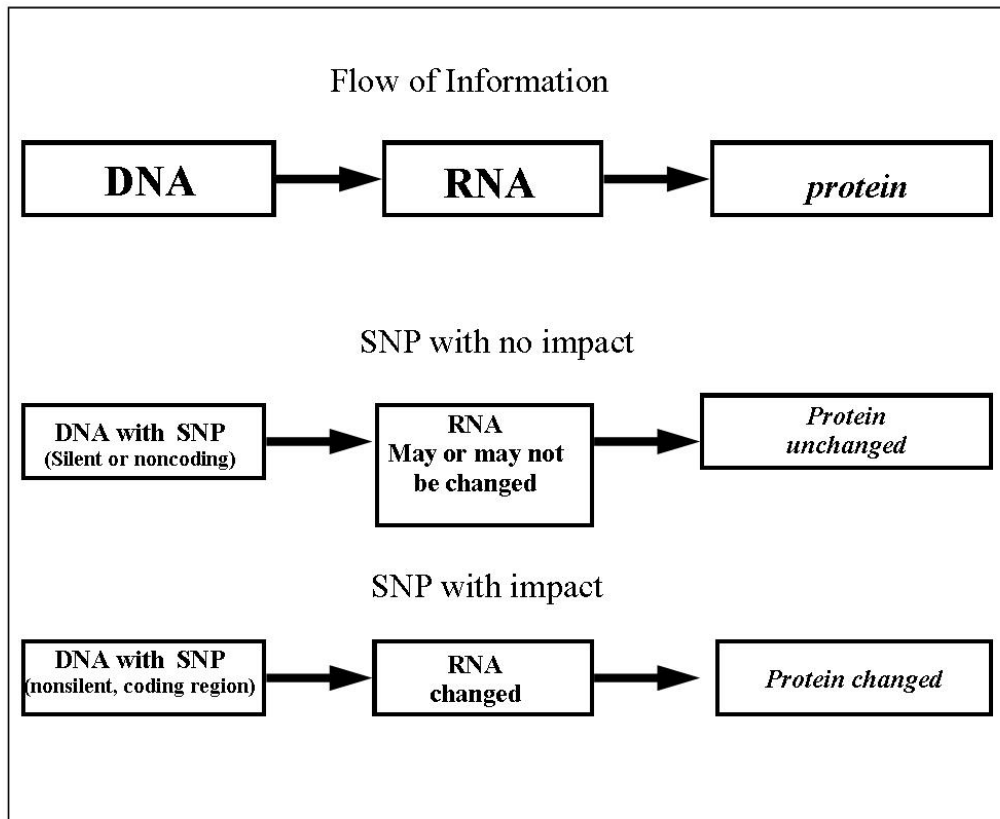
In the future, it may be possible to look at the SNPs or haplotypes in a person's DNA and tailor drugs for their illness according to their genetic profile. It may be found, for example, that people with a certain genetic profile are better at tolerating a drug than another group. However, environmental factors and people's lifestyle choices must also be considered when looking at health outcomes.

How could SNPs affect how you react to a drug?

How a drug impacts you is influenced by many genetic and environmental factors. If we focus on the genetic factors, we could find differences in genes involved in:

- Taking up the drug into the body
- Interacting with the drug (the drug 'target')
- Processing the drug for removal and removing the drug

The Leukemia case study focuses on a **cancer drug** (6-mercaptopurine) that is changed by the body into the **active form** (TGN, **thioguanine nucleotides**). **The thioguanine nucleotides disrupt cell division, which especially impacts cancer cells.** The body can also process the drug to clear it from the body using an **enzyme** (TPMT, **thiopurine methyltransferase**). SNPs in the DNA coding for the TPMT can vary between individuals, resulting in different abilities to process the drug and clear it from the body.



Examples of SNPs with impact:

Disease

Example: SNP in ApoE gene

Certain SNP variants are more likely to be associated with Alzheimer's disease.

Drug Reaction

Example: SNP in gene coding for enzyme involved in clearing drug product from body.

Enzyme characteristics are changed, and enzyme is no longer able to effectively clear drug product from body due to lower activity. Levels of the drug product build up to dangerous toxic levels.

Pharmacogenetics – Other Examples

Cytochrome P-450 Enzymes (CYP) and Drug Processing

There are over a thousand different CYPs, found mostly in the liver. CYP enzymes play an important role in helping the body to process harmful substances by making them more water-soluble. A 1997 study of over 315 drugs found that 56% of them were cleared by enzymes in this family (1). Sometimes, they play an important clinical role in converting a drug into a more active form. Many important CYP genes are found to vary between individuals (either in the number of copies of the genes or in their sequence), and some of this variance accounts for different responses to drugs. Some extensively studied CYP genes include 2C9 and 2D6. CYP2D6, CYP2C9, CYP2C19, and CYP1A2 testing is currently offered by the Seattle-based Genelex company (2).

Aldehyde Dehydrogenase and Alcohol Metabolism

Alcohol dehydrogenase and aldehyde dehydrogenase together help detoxify a wide range of organic compounds, toxins, and pollutants. Population studies have indicated that about half of certain Asian populations, including Chinese, Japanese, and Koreans have a deficiency in the enzyme aldehyde dehydrogenase (ALDH2), which processes acetaldehyde. Acetaldehyde is a product of alcohol metabolism. The mutant ALDH2*2 allele, which is incompletely dominant, reduces enzyme activity and increases enzyme turnover. Asians possessing one or more of the ALDH2*2 alleles become visibly flushed after drinking alcohol, in contrast to those who are homozygous for ALDH2*1 (3).

Herceptin® and Breast Cancer

Breast cancers are varied - each has its own set of genetic mutations, tissue characteristics, and optimal treatment. Women who have metastatic breast cancer (cancer which can spread to other organs) and also overexpress the HER2/neu gene have an aggressive form of the disease. Approximately 25-30% of metastatic breast cancer tumors overexpress the HER2/neu gene, which codes for a cell surface receptor. The receptor is thought to play a role in normal cell growth by signaling the cell to divide. When the HER2 gene is overexpressed, extra receptors are produced on the cell surface, triggering uncontrolled growth and cancer. Herceptin® is an artificially developed monoclonal antibody specifically targeted against the HER2 receptor. Herceptin® is thought to work by binding to receptor sites on the cell surface, limiting cell division and growth (4).

Warfarin and Blood Clotting

Warfarin is a widely used blood anti-coagulant. It has a 'narrow therapeutic range', meaning that the dosage is very important (too much of the drug leads to excess bleeding, too little results in blood clotting). The main enzyme involved in warfarin metabolism is CYP2C9, and research indicates that two common variant alleles (polymorphisms) of the CYP2C9 gene appear to influence patient outcomes. The *2 and *3 variants appear to have reduced activity and patients with these genotypes require lower doses of the drug (5). Recent research indicates that variants in the VKORC1 gene encoding the actual protein target of warfarin (vitamin K epoxide reductase, a protein involved in vitamin K metabolism) also critically impacts drug response (6). The VKORC1 genotype explains approximately 20-25% of variation in response to warfarin, and the CYP2C9 genotype explains approximately 6-10% of the variation. Studies also suggest that persons of Asian, European, and African ancestry tend to require on average lower, intermediate, and higher doses of warfarin, respectively.

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The following table gives other examples of inherited or acquired variations in enzymes and receptors that affect drug response

Available online at <http://www.aapspharmsci.org/view.asp?art=ps020104>

Enzyme	Variant Phenotypes	Drugs	Modified Response
Plasma pseudocholinesterase	Slow ester hydrolysis	Succinylcholine	Prolonged apnea
Acetyl transferase NAT2	Slow, rapid acetylators	Isoniazid Sal fencethazine Procainamide Dapsone Sulfasalazine Paracetamol Heterocyclic amines (food mutagens)	Slow: toxic neuritis, lupus erythematosus disease susceptibility, bladder cancer Rapid: colorectal cancer
Thiopurine methyltransferase	Poor TPMT methylators	6-Mercaptopurine 6-Thioguanine Azathioprine	Bone marrow toxicity, liver damage
Dihydropyrimidine dehydrogenase	Slow inactivation	5-Fluorouracil	Possible enhanced toxicity
Aldehyde dehydrogenase, ALDH2	Fast, slow metabolizers	Ethanol	Slow: facial flushing Fast: protection from liver cirrhosis
Catechol O-methyl transferase	Levodopa Methyl dopa	High, low methylators	Low: increased response
CYP 2D6	Ultra rapid* Extensive* Poor metabolizers	Desipramine Sparfen Phenfluramine Nortriptyline Dextropropofol, etc	Poor: increased toxicity Extensive: lung cancer? Rapid: drug resistance
CYP 2C9	Poor metabolizers	Tolbutamide, S-warfarin, Phenytoin nonsteroidal anti-inflammatory agents, imipramine	Increased response or toxicity
CYP 2C19	Poor, extensive hydroxylators	Mefenytol Hexobarbital Omeprazole Proguanil, etc	Poor: increased toxicity, poor efficacy (proguanil)
Receptors			
β_2 Adrenoceptor	Enhanced receptor downregulation	Albuterol Ventolin	Poor control of asthma
5-HT _{2A} Serotonergic receptor	Multiple polymorphisms	Clozapine	Associated with variable drug efficacy
HER2	Overexpression in breast and other cancers	Trastuzumab (Herceptin)	Overexpression associated with therapeutic efficacy
Transporters			
Multiple drug resistance transporter	Overexpression in cancer	Vinblastin Doxorubicin Paclitaxel, etc	Drug resistance

*Hyperactivity can result from activating mutations or gene duplications.

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http://www.ornl.gov/sci/techresources/Human_genome/faq/snps.shtml

Other Resources

<http://discoverysedge.mayo.edu/pharmacogenomics/>

Brief overview of the TPMT case and other pharmacogenetic examples in layperson's terms

Mancinelli L, Cronin M, Sadee W. Pharmacogenomics: The Promise of Personalized Medicine. AAPS PharmSci. 2000; 2 (1): article 4. DOI: 10.1208/ps020104

Good overview of pharmacogenetics and pharmacogenomics, with table providing examples of inherited or acquired variations in enzymes and receptors that affect drug response

Available online at <http://www.aapspharmsci.org/view.asp?art=ps020104>

http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml

General primer on pharmacogenomics.

Krynetski and Evans (1998) Cancer Genetics '98. Am J Hum Genet 63:11-16

Pharmacogenetics of Cancer Therapy: Getting Personal

More scientific background on TPMT

<http://www.journals.uchicago.edu/AJHG/journal/issues/v63n1/980379/980379.html>

GeneTests and GeneReviews

<http://www.genetests.org>

Weinshilboum, R, Wang, L. (2004) Pharmacogenomics: Bench to Bedside. Nature Reviews-Drug Discovery, 3:739-748

This comprehensive review article provides background on the development of pharmacogenetics/genomics, and highlights important aspects that need to be considered in order to bring the scientific developments into clinical practice. TPMT is a featured example.