Fundamentals of Pharmacogenomics: Origins, Definitions and Concepts
Richard M. Weinshilboum, M.D.

DR. WINN-DEEN: With that, I would like to introduce our first speaker, who really needs no introduction because he is, if I dare say it, the grandfather of pharmacogenetics. Dick Weinshilboum joins us today from the Mayo Medical School, where he is presently professor of molecular pharmacology and experimental therapeutics. He was intimately involved with the thiopurine methyltransferase research and actively teaches both pharmacology as well as pharmacogenetics within the Mayo institution.

DR. WEINSHILBOUM: First of all, let me thank the committee for the invitation. As someone who has been doing this sort of stuff for decades, to be introduced as -- I am a grandfather, but to be introduced that way is a little disheartening early in the morning.

(Laughter.)

DR. WEINSHILBOUM: So what I thought I might do to be helpful to the committee, and I think really our role here is to be helpful to you, is to do pretty much what I did with a group of graduate students for this talk yesterday morning at about the same time. So I was asked to begin with some origins and concepts, in essence a quick overview of where we are.

Let me begin with a disclosure. I'm occasionally invited, although for years I wasn't -- all of a sudden I've become very popular since the FDA guidelines came out. So I'm invited to pharmaceutical and biotech companies, but Mayo is in the upper midwest where the Scandinavians settled and were quite a socialistic institution. So all of the honoraria fees do not come to me. They go back to Mayo Foundation to support our missions in research and education.

On a very serious note, there's a flipside to this. I've spent my entire life in an academic environment, and that's why it's so important that we have Eric Lai and Walter Koch here to give you an up-close and personal view from the for-profit industry side, because their view will be quite different than mine.

I should also, in the matter of a disclosure, point out that I currently have the honor to chair the National Institutes of Health Pharmacogenetics Research Network, the PGRN, with this little logo which you'll see down in the corner of my slides, since they paid for the slides, and each of these little starts represents one of these centers. As of next week, Kathy Giacomini from UCSF will become the next chair of that group. The stars will move around a little bit, so I'll be back in Bethesda next week, where my wife says I should get a condo.

So let's begin, sort of Pharmacogenetics 101. You all know that what we're talking about is the study of the role of inheritance, that is who your mom and dad are, in essence, in variations among individuals and their response to any xenobiotic, including those that I as a practicing internist write a prescription for, the patient takes to the pharmacy, and takes the medication thinking that I know what I'm doing. So basically drugs are just a subset of xenobiotics, and we're talking about genetic variation in the drug response, in the chemical response phenotype.

In many ways this represents a confluence of two revolutions, that is the genomic revolution which everyone who reads Time magazine knows about, but as a matter of fact I feel very strongly as a pharmacologist that in the latter half of the 20th and the beginning of the 21st century there has been a parallel therapeutic revolution in which we have gone -- and I like to

demonstrate this for my medical students in this fashion. This is the first edition of Goodman and Gilman's textbook, 1941. I was actually around then, but rumors among the male medical students to the contrary, I was not reading G&G then. Here is the 10th edition. The books are the same size. There's virtually nothing in this book. That is, there is morphine and there's digitalis, there's aspirin and sulphur drugs. But no antibiotics, no antihypertensives, no antipsychotics, no antidepressants. Franklin Roosevelt was president of the United States and had hypertension, was treated with phenobarbital, which made his doctors feel better but didn't do much for his blood pressure.

So as a matter of fact, there has been a dramatic change in the therapeutic agents which we have available. I think it's been a quiet revolution, but as a matter of fact it's been earth-shaking. We talked about paradigm shifts in your introductory comments. Bring that together with the genomic revolution, and those are the ingredients that have created what we are talking about today and is the reason basically that we're sitting here, because the concepts of pharmacogenetics and pharmacogenomics really date back half a century. Every time I'm called up, as I was by Public Radio the day before yesterday, and they say Francis Collins thought this up, well, Francis is a wonderful man, but he didn't think this up. As a matter of fact, these concepts have been around for half a century, but they have been accelerated dramatically by the technology that came out of the Genome Project.

So my definition of pharmacogenomics is the convergence of the advances in pharmacogenetics that have occurred over decades with the striking progress that has occurred in human genomics. You bring that volatile mix together and I think that's one of the reasons that we're sitting here.

The clinical goals are obvious, and in the introductory comments we mentioned avoiding adverse drug reactions, and I'll use an old chestnut, namely TPMT, to illustrate that in just a moment. But let's don't forget that we're also maximizing therapeutic efficacy, selecting those patients who might respond best to the drugs. Frankly, one of the impediments, and I'm speaking now from the view of the academic world, to the involvement of pharmacogenomics in the drug development process has been this issue of selecting responsive patients, which limits the markets for the drugs. Now, I'm sure I'll hear something quite different in just a moment, but we need to get the issues out and at least talk about them here.

The scientific goals are also obvious, the correlation of variation and DNA sequence or structure with variation in the drug response phenotype, the so-called genotype/phenotype correlation. Now, I never thought in my lifetime, and I've been doing this stuff for over three decades, that I'd be standing here talking to you about DNA sequence. As a matter of fact, the postdocs in my lab, I walked in the other day on a Sunday and I said, okay, Ezekiel, how many base pairs did you sequence this weekend? He said 5 million. This is a mom and pop store, folks.

So when you stop and think about that, that's truly an amazing revolution that has occurred. Let's immediately say -- I mentioned that I'm an internist -- that all of us who write those prescriptions understand that genetics are only one factor that plays a role in individual variation in drug response. The patient's age, renal function alters rather significantly with advancing age. We are increasingly sensitive to the fact that males and females respond differently to drugs. Underlying disease and drug interaction all play a role. So this is only one factor, but it's one where objective information may now be brought to the physician, and the challenges which you mentioned in your introductory comments, how do we help the practicing physician to integrate this information into the therapeutic encounter, is going to be an interesting challenge.

Let's don't forget, because my medical students do, they focus on what does the drug do to the patient, but the patient is doing a lot of things to the drug. That is, the drug must be absorbed, and we know the transporters play a role in this process, get to its site of action, interact with its targets, be metabolized and excreted. All of these processes, we now know, have very significant and clinically relevant genetic variation. Most of this field grew out of the field of drug metabolism, but that's only as a demonstration project because of pharmacokinetics we could gain insights into intact, unhomogenized human beings by looking at pharmacokinetic parameters and therefore look at drug metabolism.

I like to think of this as a scientific evolution analogous to the way in which we have approached the application of genetics to diagnostic medicine. Let's begin with some rather dramatic monogenic traits, and I'll show you some of those examples in just a moment. They were necessary to make the point, because I can't tell you how many years I would go around to departments of pharmacology talking about pharmacogenetics, and as soon as I'd say the words "allele" or "polymorphism," everyone's eyes would glaze over, their palms would get sweaty, and nobody would pay any attention.

Then they would tell me, why don't you get a nice inbred mouse because they won't show this yucky variation. And I would say I'm studying the variation. So we had to make the point, and TPMT and CYP2D6, if they didn't exist, we would have to invent them, and I'll tell you about them in just a moment. But that will not be probably an example of the major way in which genetic variation will manifest itself. Increasingly, we're talking in terms of both PK and PD pathways, and I'll define those in just a moment, and increasingly adding genome-wide screens at the scientific level to gain insights into the myriad ways in which genomics can play a role in individual variation in drug response.

Pharmacokinetics -- and I'll just in the remainder of my comments talk about PK and PD -- are those factors that influence the final drug concentration at its target, predominantly transporters, drug metabolizing enzymes. Pharmacodynamics are those factors that influence the response of the target itself, not just the target but all the downstream signaling that comes from the target. We now know that although we might be able to make an end run around this, it's going to be awfully hard to make an end run around genetic variation in the pharmacodynamic pathways.

Now let's use a couple of what Eric turned to me and said I assume you're going to talk about the old chestnuts, and I said yes, sure, of course I will. So let's use these two, and I like to use them because they're both well validated, and because in the draft pharmacogenomic guidance that the FDA put out in 2003, and I guess in March of these year these are no longer draft, they selected these two, thiopurine methyltransferase, TPMT or CYP2D6, as valid biomarkers, meaning they're old fashioned and we all know a great deal about them. So let's use TPMT as a prototypic example.

Here are the thiopurine drugs, 6-mercaptopurine, which was developed in what was then the Burroughs-Wellcome company by George Hitchings and Gertrude Ellen. They shared the Nobel Prize in 1988 in part for the development of these drugs which are a mainstay in the treatment of acute lymphoblastic leukemia of childhood, a disease that was uniformly fatal when I was in medical school, and today we cure 85 percent of these kids with drugs -- no surgery, no radiation therapy. That's what I mean when I say the therapeutic revolution was a quiet revolution. These drugs were also used as immune suppressants, azathioprine, which is just 6-mercaptopurine with

amanadazol up here, which is cleaved off in vivo, and they're used in the treatment of inflammatory bowel disease.

Now, even the Mayo medical students who I teach know that these drugs are metabolized by xanthine oxidase. George Hitchings and Gertrude Ellen knew that they also underwent a so-called phase II conjugation reaction where a methyl group was stuck on that sulphur. The metabolites were present in the urine. Twenty-five years ago, no one knew anything about the variation in the enzyme itself, but these are very powerful cytotoxic agents, and every now and then you would treat one of these children with leukemia and the drug would destroy the child's bone marrow, and the child would die from the drug therapy, not anything that anyone wanted, what we would have referred to in those days as an idiosyncratic reaction, which means we don't understand what the cause is.

This just shows you data which we published 25 years ago now on TPMT in the human red blood cell. In case I forget to say it, what you see here reflects the level of the enzyme activity in every human tissue, for reasons that will become clear when I show you the gene in just a moment. These are 298 randomly selected Northern European blood donors in Minnesota. There's an important reason why I say that, and I'll come back to it in just a moment. That is, everyone in Minnesota, except me, is named Anderson and Johanson and stuff like that.

But there's a scientific reason for bringing that up. Ninety percent of this population had high activity, about 10 percent had intermediate activity, and this lady down here, whose daughter works at Apache Mall in Rochester, Minnesota, had zero enzyme activity. Rochester is a very strange town, folks. People will stop you when you're walking through the mall and ask you how your mom's enzyme activity is doing.

So using very, very sensitive molecular techniques developed by a monk in a monastery in what is today Brno in the Czech Republic -- this was before anyone had cloned much of anything. So we were using segregation analysis. If mommy is low and daddy is high, what are the kids? You could just as easily determine that this was a genetic trait using that approach. You can say that this woman has two copies of a gene for low activity, these people have two copies of an allele for high activity, and these are heterozygous with intermediate activity, and autosomal co-dominant trait, which is true for every tissue. This just shows you the consequences of having two copies of low. This was long after Lynn Leonard and I had described that if you have low TPMT activity, you are at serious risk for life-threatening myelosuppression.

This is a heart transplant patient in Germany treated with standard doses of azathioprine. Here's the white count. Here's the azathioprine dose. Notice that the white count drops, the drug is stopped; it goes up, the drug is started. The white count goes down to zero, the drug is stopped. Started again. The patient died here with myelosuppression. They then measured the TPMT in the red blood cell. This patient genetically lacked the enzyme.

These cases, by the way, are not reported any longer. Do they occur? Tragically, yes, because I get many of the telephone calls. I got one just two weeks ago, again exactly the same situation.

So if you have low TPMT activity on a genetic basis, you're at greatly increased risk for thiopurine toxicity, which can be life-threatening. Mary Relling at St. Jude has demonstrated this is also a risk factor for secondary neoplasm. When we cure these kids for their primary neoplasm, Lynn Leonard in Sheffield has shown that high TPMT, you have decreased therapeutic efficacy for a life-threatening disease. At our place we have been doing the TPMT genotype, and

then the phenotype study, since 1991. We do about 5,000 to 10,000 of these tests per year, about half on our own patients and about half referred in from physicians outside, and we are individualizing therapy. Clearly, if we see these people, we treat them with one-tenth to one-fifteenth the standard dose, and that's been our situation for about 15 years now.

The cDNA was cloned by Ron Honshal in our lab, who is now at the FDA. The gene was cloned by Diane Otterness, who is out in California. Here's the gene itself. It is 10 exons, eight of which encode protein. On the short arm are chromosome 6. The blue area here is the part that encodes the protein. The most common variant allele in Caucasians, which we described in 1996, has two non-synonymous coding SNPs that change the encoded amino acid 1 on axon 7 and axon 10. If you have that variant, which is present -- this is not a mutation. This is a common polymorphism, the frequency is one out of every 20 copies of that allele in Northern European Caucasians -- then you are at very greatly increased risk for drug-induced toxicity if you're treated with standard doses of thiopurines.

By the way, that variant allele has never been described in anyone from Korea, Japan or China. That was the reason I made the point, and we're going to come back to this in my later presentation, and one of the reasons I was called by National Public Radio was to ask about BiDil. The hearings are today, so I think we'll be coming back talking about that. This is the variant that's found in East Asia. It just has the axon 10 variant at about a 2 percent frequency.

Because of the dramatic clinical consequences, and because it's relatively well validated, this was one of the first examples that the Food and Drug Administration considered for possible inclusion of this information. Labelling had two public hearings. I testified at both of them. Felix Frueh is here. I saw him before we began. That was an interesting experience which I'm sure he'll describe in greater detail.

Let's move on to CYP2D6 to give another example. It's the same song, second verse. Interestingly, we published our first paper on TPMT in 1978. It was the assay that we knew we wanted to use for pharmacogenetic studies. It was almost at exactly the same time that the first paper on 2D6 was published. So these are old examples, folks, and that's why Eric asked me, oh no, am I going to have to hear about TPMT and 2D6 again? So this just shows you that cytochrome P4502D6 metabolizes 40 or 50 commonly used drugs, including beta blockers and antidepressants.

Here you're looking at a metabolic ratio for the antihypertensive dubresoquine, which was never introduced on the market in the United States. It undergoes 4-hydroxylation catalyzed by 2D6. Counter-intuitively, the way we have represented this, the way pharmacogeneticists do this is to show the metabolic ratio. These are the poor metabolizers up here. It's about 5 to 10 percent of a Caucasian European population. Once again, I say that because there are ethnic differences in allele frequencies and types.

This group is the extensive metabolizers, and these low numbers are ultra-rapid metabolizers. That obviously is also -- or not so obviously but also of clinical importance.

This just shows you data from -- the previous slide came from the Karolinska, from Lief Battleson's lab. This is also from Lief Battleson's lab at the Karolinska, where they're looking at the tricyclic antidepressant nortriptyline, and what you're looking at is pharmacokinetics -- that is, plasma levels over time -- depending on the number of active CYP2D6 genes that you have. Most of us have two copies of that active gene. Here is our pharmacokinetic profile. By the way, this slide unites the two topics which are the least favorite of the male medical

students. They find drug metabolism boring. They find pharmacokinetics terminally boring. Putting the two together here in one slide is amazing.

So you can see if you have two copies of a variant, you can either have gene deletion or you can have polymorphisms that result in no activity. You have a much higher peak plasma level and a much larger area under the curve. But look down here. This lady, who was herself a nurse at the Karolinska, had 13 copies of the active gene. Look at her pharmacokinetic parameters. Now, her metabolites were way up there, way off scale. So these are active genes. This just shows you what can happen.

In most cases, CYP2D6 terminates the action of the drug. But for codeine, what it does is activate it by converting codeine to morphine. So if you are a poor metabolizer for 2D6, and that's 5 to 10 percent of the European population, you will not get the analgesic effect from codeine. But if you're an ultra-rapid metabolizer -- and this was a very recent case report in the New England Journal, December 30th, 2004. Sixty-two year old man hospitalized for pneumonia, treated with standard doses of codeine, right out of the PDR, as a cough suppressant. The next stop was the ICU because the patient stopped breathing. He had morphine levels 20 times the expected level. He was an ultra-rapid metabolizer.

I just show you this as a preview of Walter. I have no stock in any company, and certainly not in Walter's, but let me say that all that we're doing here is using this metabolic ratio to give us insight into what's going on at the level of the DNA. In today's world, and we'll be talking about this later, devices like the one which comes from Roche Diagnostics, give us direct insight into the DNA.

I finally want to give us a peak at the future. I feel obliged. I live in Minnesota. We're right next to Wisconsin. This is Karl Paul Link, the man who discovered warfarin, an amazing person. If you haven't read the story of the discoverer of warfarin and the farmer with the bucket of blood in the Wisconsin blizzard, go back and read it. They don't let you write articles like that anymore.

Warfarin can occur as an S and R antimere. The S is metabolized by CYP2C9. This just shows you that warfarin blocks the Vitamin K pathway which is required for the gamma-carboxylation of glutamic acid to make active clotting factors. The epoxide reductase shown in this little cycle here was only cloned just about a year ago. First let's look at the metabolism.

So now we're looking at the PK, the pharmacokinetic pathway, and there are common genetic polymorphisms for cytochrome P4502C9 in European populations. If you're homozygous for the *3 variant, you can see the clearance is much reduced as compared to the clearance of S-warfarin, which is really the most active portion of the warfarin. Here you can see what we see in the individuals who are homozygous for wild type 2C9. But look at that variance. Big variance.

Now we're looking at the Vitamin C cycle, and it was in Nature, February 5th, 2004 that this target was first cloned. You would think we would have known about it before then, but we did not. I assigned this for our journal club. The people in my lab said wait a minute, we don't do warfarin stuff. Why are you assigning us this? I said because somebody is going to resequence this gene in about 10 minutes, and when they do, this will be used for pharmacogenetic research. Several groups did.

This is from the June 2nd, 2005 New England Journal. National Public Radio asked about this, too. So they're becoming very onto pharmacogenetics. That gene is called Vitamin K oxidoreductase C1, or VKORC1. The gene was resequenced. Ten common SNPs and 5 common

haplotypes were identified. None of them were non-synonymous SNPs. They didn't change the encoded amino acid. So now we're moving on to the world of haplotypes, the combination of SNPs on a given allele. They divided their groups into low-dose and high-dose haplotypes.

Notice the mean maintenance doses of warfarin, about 2.7 for those who had two copies of the haplotype for low dose, and 6.2 for two copies of the high dose. This variant was responsible in their studies for about 30 percent of the variation in final warfarin dose, CYP2C9 about 10 percent. You begin to put those together and now you're beginning to talk about something that, if you're prescribing warfarin, you might want to know about.

So the scientific evolution -- and I'll try to keep us on time -- was monogenic traits. Pathways were increasingly incorporating genome-wide screens and scans. Let's don't forget what the clinical goals are, not only avoiding adverse drug reactions but probably over time, more important, maximizing efficacy and selecting responsive patients. That has pharmacoeconomic implications which I'm sure you'll want to discuss later.

Let's don't forget the scientific goal, because as the science rolls forward, our ability to bring ever more complex, ever more complete information to the bedside is going to accelerate, and the vision, which we will never achieve -- I understand that. I'm a practicing physician. But the vision is very clear, to select the right drug at the right dose for every single patient that we see.

Thank you very much. I hope this is helpful.

(Applause.)