Pharmacogenomics in the Practice of Medicine
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DR. WINN-DEEN: So we're now ready for Weinshilboum Part 2. Now he's going to focus a little bit more on his role as a physician and talk to us about pharmacogenomics in the practice of medicine.

DR. WEINSHILBOUM: And what I'd like to do now, and I've now got a lavaliere and I've got a really fancy laser here, is to move beyond the sort of Pharmacogenetics 101 and begin to talk about the issues which we appropriately have already begun to talk about; that is, the translation of this information into the clinic. But I think we need to step back, and I've called this "Challenges and Opportunities." Dr. Davis had something similar.

As I thought about how to organize this, I think it's important to talk about it in terms of the science, and I've divided it into basic and translational science, drug development and regulatory science, and ethical, legal and social science, about which I as a pharmacologist am clearly a novice. But I think it's important to put up a diagram like this which we already have implicitly talked about, and that is eventually what we want to get to is the therapeutic encounter between the physician and the patient when either the physician writes the prescription or, as Dr. Davis said, HAL the computer writes the prescription, whatever we end up with so that the patient has the right drug at the right dose.

In general, those of us in academic centers tend to think in terms of academic medical centers, like Mayo or Duke or whatever your personal one happens to be, and a relationship with our funding agency -- it can be American Heart, NIH, et cetera -- and that we will be able to influence this in some fashion.

That's a short-sided approach because, frankly, drug development in the United States since the Second World War has focused on the pharmaceutical biotechnology industry, and just as the NIH is the place that predominantly those of us in academic centers look to, we need to think in terms of regulatory agencies, and particularly the Food and Drug Administration.

Now, interestingly, the amount of interchange between these groups -- that is, between, say, the NIH and the FDA, speaking totally as a novice, so just as I made the point initially that I spent my life in an academic medical center, I clearly know nothing about this area other than what I found as a tourist dropping in to give a lecture every now and then. But it struck me that these two agencies didn't talk to each other that much in the past. What you're going to hear is that that dialogue is also important, and we're moving forward with regard to those kinds of interactions. That's already been mentioned in previous presentations.

So let me begin by pointing out that although our focus has been on translational pharmacogenomics, Dr. Long from the NIH is here, and she would point out that NIGMS has been supporting our research for 30 years, and clearly we need the basic pharmacogenomic research in order to get to the translational research, and they feed off of each other. I think it's important to make that point because Dr. Davis was talking about putting his teams together.

Frankly, we have found for our teams, which include molecular epidemiologists, population scientists, clinical investigators, that having basic scientists involved is critically important, because what happens is the basic science runs right by what you're doing. It says goodbye to it and runs right by it. So we need to be sure that the latest developments are incorporated in this, and the whole team really includes all aspects of health care research.

I want to come back to the scientific goal because we were just talking about the National Human Genomic Research Institute and what they can offer, and obviously our understanding of the genome keeps changing right beneath our very feet. So the nature of sequence and structure differences in DNA that can have practical implications at the translational interface keeps changing. This is a slide that I keep adding to with regard to the nature of the sorts of genetic variation that will be important and is important in pharmacogenomics.

Obviously, the SNPs, the single nucleotide polymorphisms, the insertions/deletions, VNTRs. Gene deletion and duplication I already mentioned with regard to CYP2D6. Increasingly, we are finding large segmental duplications, and I'll actually show you an example in just one second. So the nature of the kinds of assays we have to do keeps changing, and that, Dr. Davis, is why I said you need the basic scientists sitting right there, in person, in the flesh, at the table, because your assays will be out of data mañana. Gene variation resulting in alternative splicing. Whole new areas of genomic science are opening up, and epigenetic or what I like to call pharmaco-epigenetic variation.

I'll show you just this one example. What this is showing you is on chromosome 16, a duplication of 145,000 base pairs, one of the genes we were studying. The idea of the Genome Project being "complete" is an interesting and ever-changing target, but this area has one of our genes that is 99.9 percent identical, duplicated right in the middle of this duplication of this big chunk of DNA. Well, that really messed up our genotype. The comment was made, what about sequencing? Well, sequencing, even if you're using dye primer sequencing, if you've got instead of two copies of that allele, four copies, and you're trying to interpret your sequence traces, that's a real mess. I won't bore you with the details other than to say the science is changing out there, and we need to remember that the basic science is going to drive this process, too.

At the NIH -- and I put this within the context of the NIH Roadmap. So the director of the NIH and the NIH has gone through this strategic planning exercise in which they have given it the usual strategic planning catchy phrases, but the concepts are pretty simple. New Pathways to Discovery means biology is very complicated, and no one has the expertise to know all aspects of it, so you need the kinds of teams that Dr. Davis was talking about at both the basic and translational level.

The Research Teams of the Future means that you're going to have to organize the way in which we gain the new knowledge and test the knowledge in new and different ways. Now, I've never done any knockout mice, but if I could do a human knockout, there's really only one gene I want to knock out, the gene for the human ego structure, because, frankly, the biggest barrier to putting these sorts of groups together is who is in charge here, and we need to find ways that we can adequately reward team and social interactions in ways that our current system frankly discourages.

Finally, Reengineering the Clinical Enterprise basically is the need for multi-center, multi-group organizations because of just what Dr. Davis was talking about. The power calculations are going to kill you, and no place -- the Mayo Clinic is a big place, but we know that we have to team up with other institutions in order to be able to have adequate numbers of patients to test these hypotheses and determine how we want to move forward.

What has happened as a result of -- and I got in a little trouble with Tim about my comment about Francis Collins not thinking up pharmacogenomics. But what's happened as a result of the dramatic changes that have occurred in genomic science is that whereas the examples of TPMT and CYP2D6 began with phenotype and with armies of postdoctoral fellows shoulder to shoulder

across the world marching out, they purified the protein and cloned the cDNA and cloned the gene -- I even told you the names of some of them -- got the polymorphism, and that took 15 or 20 years, in today's world we type "NCBI" into our web browser and then you've got the gene sequence. That was what Dr. Honshal spent a year and a half of his life to get.

So now we can begin with genotype and go back to phenotype, and one of the complementary strategies that's being used in this area is to very rapidly determine gene sequence variation in individuals of differing ethnicity. Once you have the common variation in gene sequence, then to do the functional genomics to determine which of that variation is functionally significant, and then the really hard part which Dr. Davis was talking about, to determine which of the common variation that's functionally significant is of clinical importance. Those are among the challenges. This is not the only way to do it. Genotype to phenotype and phenotype to genotype are complementary approaches.

Let's take a different example. I made an interesting observation myself when I put these examples together. 2D6, TPMT, warfarin, 2C9, VCORC1. I said where has this information come from? There's an important point here, and I'm challenging Walter and Eric because all of this information, all of these chestnuts have come from academic medical centers. They have not come from industry. The challenge, Eric, for industry is to find ways that we can partner with our mutual strengths in order to be sure that in the future industry is making -- I'm being a little provocative here, and that's unusual for me, but let me do it anyway -- that industry is making these kinds of contributions.

So the irinotecan example. Irinotecan is an antineoplastic agent, a camptothecin derivative. It inhibits topoisomerase I, and its toxicities are predominantly diarrhea and myelosuppression. This diarrhea is not just something that you take a little Imodium for. This is life-threatening diarrhea.

Here's the way that, now going back to boring drug metabolism -- irinotecan itself is a pro-drug. It's metabolized by cardoxylesterase to form SN38, which is the active drug, which is itself glucuronide conjugated by UDP glucuronisil transferase, and that gene -- I have to show these gene structures because I love them. This is a really nice gene that I love to tell the graduate students about. It has a whole bunch of upstream exons that are then alternatively spliced in to conserve four downstream exons, and then you get the substrate specificity depending on which of these you set in.

Well, the one that metabolizes irinotecan is UGT1A1. That is also responsible for bilirubin metabolism and for Gilbert's syndrome, not disease but syndrome. We now know that that's predominantly due to variable number 10 and repeat in the ta-ta box. If you have seven ta's, you have a lower level of activity. This is in the promoter. If you have six, which most people do, you have a higher level in people who are homozygous for seven, like myself. Every time I go in for my physical exam, I'm told by the intern or resident who is doing the exam, well, your unconjugated bilirubin is up a little bit, and it always is when I'm fasting. That doesn't make any difference in most settings, but with irinotecan, it makes a big difference because that's the isoform that metabolizes irinotecan, and if I'm ever treated with that drug, which I hope I never need to be, I know that I will need a somewhat different dose, a lower dose of the drug.

This is to get us to the pathways. It's also to do something else. Here's irinotecan. This is from the pharmacogenomics knowledge base, PharmGKB, which is sponsored by the pharmacogenetics research network that I mentioned, and what we're doing is putting a bunch of pathways there. All the little squares that are sort of this purple color are drugs that are

metabolized. All the little egg-shaped things are genes encoding proteins that either metabolize the drug or transport the drug, and now this begins to give you some idea of the degree of complexity that we will find ourselves dealing with with most drugs, where the metabolic and transport pathways look like an explosion in a spaghetti factory.

So you're going to find that this will become extremely complicated, and the examples that we've used are examples of simplicity. Where the world is going to take us, the real world is going to be much more complex than that. I showed you that because I wanted to be sure that I brought to your attention the fact that the NIH is sponsoring this knowledge base, PharmGKB, where all of the data from the network, and we hope from outside the network, will eventually come together in one place, genotypes and phenotypes. That kind of a database is a tremendous challenge. To try to combine genotype and phenotype, it makes GenBank, with all due respect, look fairly straightforward and simple.

So I want to talk about pathways. Having talked to medical students and graduate students forever, I've learned that reiteration is an important part of the pedagogical science, so let's go back to TPMT and let's talk about thiopurine metabolism and metabolic activation pathway, because azathioprine is a pro-drug that's converted in vivo to 6-mercaptopurine, which can be methylated or oxidized. That's kind of what I showed you a moment ago. But 6-mercaptopurine is itself a pro-drug that undergoes a series of metabolic activation steps to form 6 nucleotides which are incorporated into DNA, and that's a major mechanism, the major mechanism probably, for the cytotoxic effects of these drugs.

I show you this because this is kind of a moo cow/bow wow pathway, really. It's much more complicated than this, but I'm showing you the very simplified pathway. When we first published our data on TPMT, I will tell you that everyone knows that this is the major metabolic pathway. This is actually a minor pathway. I thought about bringing along the line from the reviewer for Cancer Research that said these dumb pharmacologists aren't smart enough to understand that this minor pathway couldn't possibly influence individual variations in response to these drugs.

Now, everybody has those sort of letters. I didn't bring it along. What was going on at that time was Lynn Leonard at Sheffield had demonstrated that by measuring 6-thioguanine nucleotides, she could predict who was going to get toxic on these drugs. She met me at an international meeting and she said, Dick, what I can't figure out is we treat these kids with exactly the same dose of exactly the same drug. Some of them will have very high 6-thioguanine nucleotide levels and some of them won't. I said, Lynn, maybe it's because this pathway genetically, if it's impaired, you pump more of the drug down here and you're going to have higher 6-thioguanine nucleotide levels. So she sent us blood samples from 95 consecutive children in the U.K. who are in the UKAL, the United Kingdom Acute Lymphoblastic Leukemia trial.

We measured the enzyme activity, she measured the 6-thioguanine nucleotide levels. When you got up here to 600 to 800, that's when you begin to have myelotoxicity, and these are the heterozygous individuals. She also had samples -- these are data we published in 1989 -- samples from individuals treated with standard doses of these drugs who developed life-threatening toxicity. Half of them died. She sent us those samples and a group of controls. These were patients with dermatologic disease being treated with azathioprine. Notice we're up in the thousands of picomils for the active metabolite. This person was 26 days after the drug was stopped and he was still above any of the controls on the same dose of the drug.

When we published this, we said if this can be confirmed, we can predict and prevent this toxicity, and indeed it's been confirmed, as I mentioned, over and over and over again. But that's to make the point that pathway analysis is extremely complicated, and what you think a priori, just because something is a major pathway, like the xanthine oxase, doesn't mean that's going to swing the variation. So the translational lessons for TPMT, among others, are the importance of having an intermediate phenotype like the 6-thioguanine nucleotide levels. Kids with leukemia are treated with a large number of cytotoxic agents. There are a variety of reasons why they are going to become myelosuppressed. If they have a viral infection while they're on these agents, they will have myelosuppression. But by having the active metabolite, we can sort out those in which it was the TPMT that was the problem.

In addition, it emphasizes the difficulty of pathway analysis. So when we design these studies, the mega-study, the 100,000-patient study, we need to understand that it's going to be extremely difficult to fish out what a given genetic variation might be doing of importance.

This is just to make the point that the modified central dogma is not gene goes to mRNA goes to protein goes to metabolite, but that we now have genomics, metabolomics, et cetera, and that means that the assays that we have available will have to be very different kinds of assays. So the clinical assays will involve phenotypes, and by that I mean the endpoint, myelosuppression, or the intermediate phenotypes, and those intermediate phenotypes may well be a metabolomic signature. So it may be measuring 10,000 metabolites and using informatics to fish a signature out which at first we won't even understand. But we need to know that during the discovery phase we'll be looking at all kinds of phenotypes between the DNA and what we see in the patient. It's going to become very interesting, but I think we're going to need those different phenotypes.

At the clinical level we'll be measuring not just SNPs but also haplotypes, and eventually Tim was already talking about 3 billion nucleotides, and I'll be interested in how our doctors at the Mayo Clinic deal with that when their patients come in with it. Obviously, we'll be talking with Walter in just a moment with regard to the development and validation of these tests, significant challenges which you know a great deal more about than I do.

This is just to make the same point I made before. Walter will be talking about it, and I knew he was going to be here, so I used his device as an example. The scientific evolution here, let's think about what I've been saying and what we all know, and Dr. Long, who is in the audience, will be saying. We've gone from phenotype to genotype to a complementary genotype to phenotype, which frankly has accelerated the process 10-fold at least. So we resequence these genes, do the functional genomics, and before we even have the paper off on the resequencing data, we'll be dealing with our clinicians in the breast cancer clinic because they have the DNA to test hypotheses.

So the basic science crosstalk with the clinical science, in theory we ought to be breaking down those barriers, and with the right organizational structure, and with the diminished ego structure, we can actually get there. We've gone from monogenic traits -- clearly, that irinotecan pathway was there to say we need to be thinking polygenically, and we've gone from single genes and proteins to entire pathways, from single polymorphisms to haplotypes, genome-wide screens, and Tim will eventually give us all 3 billion nucleotides, and from the mom and pop store approach, which is what I've done through most of my career, to high-throughput platforms and groups. We've already talked about all of this. I'm just reiterating themes that Dr. Davis introduced.

With regard to drug development regulatory science, I feel obliged to put this up so poor Eric can respond to it. This is not my comment. It's from "Surviving the Blockbuster Syndrome" in Science last year talking about pharmacogenomics and that there has been some skepticism with regard to segregating out different patient populations who respond.

Now, when I do my clinical work, I work in a hypertension clinic, even the Mayo medical students, God love them, know that it's beta blocker, diuretics, ACE inhibitors and calcium channel blockers. That's not the question. The question is for whom? Which one will respond? There we're not talking about life-threatening situations all the time, but we're talking about churning the system. So they keep coming back and, oh, it didn't work, and what are we going to do, even if we have the nurses doing it. We know that about half the patients won't respond to any of those drugs.

And that brings us back to this little diagram that I showed at the beginning. Clearly, with regard to the drug development process, the role of the Food and Drug Administration and the regulatory science becomes absolutely critical, and I made a joke about this at the beginning, but as a matter of fact it was not a joke. It was true. I have noticed that since Larry Lesko and Janet Woodcock have taken an interest in pharmacogenomics, and I've got one of their papers here, and we'll be hearing from Felix about this later on today from the Food and Drug Administration, that since the FDA has been interested in this area, the pharmaceutical industry's interest has been increased.

There are tremendous differences among companies. Please, you can't generalize. But as a matter of fact, there was and remains some resistance to thinking about issues of segmentation of the market as a result of knowing at the front end which patients will and will not respond to a given class or specific drug agent.

At the translational science, we already talked about this. The involvement of this science in the drug development process is already going on. I know that. It is increasing. What that says is that all the examples I've given you -- thiopurines, irinotecan, warfarin for God sake, that's the 1930s -- these are all examples of drugs that were out on the market and academic science studied them and came to the conclusion that there were large genetic variations in their side effects or in their therapeutic efficacy.

Eventually, a great deal of this science will be built right into the drug development process. That has very significant regulatory and economic implications which I'm not qualified to deal with but which I'm sure we need to address.

Clinical trials are going on. Type "clinicaltrials.gov" into your web browser and go and look at the clinical trials, tens of thousands of them, and how many of them have pharmacogenomics built into them at the front end. Remember, you've already spent the money -- this is the point that Dr. Davis was making -- to create the infrastructure, to recruit the patients, to get the clinical data together, and you're drawing blood samples to send them off for an SMA-12 or whatever that's called in this day and age. So why don't we make DNA a part of that so that you can either prospectively or retrospectively go back and ask the questions Dr. Davis wants us to ask?

Part of the Roadmap was public/private partnerships. Within the Pharmacogenetics Research Network, we have been grappling with that. There are very significant issues of intellectual property and proprietary interests which stand as barriers, and we might as well just put all these issues out on the table so we can talk about them in the course of the day.

So we need to find ways that we can not just talk about this but actually find ways to deal with the unique problems of each side so we can deal with it.

Finally, legal, social and ethical issues. You know much more about this than I do. Confidentiality is just as big an issue here as it is with all other areas of DNA testing, insurance perhaps a little less so because nobody knows, although we have tried, what TPMT is there for. It's found in bacteria, but we don't have any disease that if you are like that lady whose daughter works at Apache Mall and comes up and asks me about mom's enzyme, who has zero TPMT, we don't know that this means you're at risk for any disease. If we ever find that out, then this becomes an issue. But for many of these variants, that's less of a problem here, although it's still a problem.

Finally, what do I mean by "therapeutic activism"? This is not like BRCA1 or 2. If I find that a patient is homozygous for low TPMT, I want to lower the dose of the thiopurine. I can do something right then, either use the drug or don't use the drug, lower the dose or raise the dose so that in this situation there isn't therapeutic nihilism. If there's ever going to be a place where there's therapeutic activism, it is in the area of pharmacogenomics.

Finally, the issue that was raised just a few moments ago. This is from the New York Times October 10, 2004, "The Genome in Black, White and Gray," and what was the focus? It was entirely on pharmacogenomics. The issue related to the hearings today on BiDil, the drug that is being evaluated for the possibility of being approved for only one ethnic group, for African Americans, is being discussed right here. I heard Francis Collins interviewed on Public Radio about that and heard his comments, which is that this is undoubtedly -- it's not skin color that's the issue but it's the underlying genetic variation, which showed these striking differences that I mentioned.

This keeps coming up. This is 2001 in the New England Journal of Medicine, where there were articles about ethnic differences and response to angiotensin-converting enzymes, and two editorials taking the kinds of diametrically opposed points of view that this committee knows much more about than I do. Here we are in 2003, New England Journal of Medicine, and it was deja vu all over again. We were having exactly the same discussion, and I come back to this just to point out that this common variant which is found in Caucasian Americans is not found in Asians.

When I was a visiting professor at the National University of Singapore, where the population is 80 percent Chinese, they said, Dr. Weinshilboum, this is a problem we see only with these European kids. What's the deal here anyway? They actually have developed the testing to use for Europeans. They clearly were devoted hematologists and oncologists that came to Minnesota in February to learn the techniques.

Finally, this issue of health care professional educational. I heard what Dr. Davis said. The implication was pretty clear, and I will have to say that in a review that Li Wae Wong and I wrote in Nature's review of drug discovery, we said that this would be an important part of what we need to do. We were roundly pilloried by the sociologists at Cold Spring Harbor. I continue to believe, because what I've seen is, at our place the gastroenterologists, who see a thousand new inflammatory bowel disease patients per year, have totally embraced TPMT; that in hematology/oncology, the resistance is basically one that in that community toxicity is their business. Push the patients to toxicity.

So we need to realize that there are sociology differences within medical subspecialties, too. But if gastroenterologists are educable, I think there's hope for everybody.

(Laughter.)

DR. WEINSHILBOUM: Finally, I want to end where I began, by pointing out that this is only one factor among many factors that influence individual variation in drug response. The clinical goals are ones that no one can argue with. No physician wants to harm his or her patient. We all want to maximize efficacy of these drugs that come out of the therapeutic revolution, and it would be much, much cheaper if, at the front end, we could select the responsive patients. Genetic inheritance is only one factor in the drug response phenotype, but the pace of our understanding is increasing dramatically, and the goal has already been demonstrated. We have examples out there that make it very clear that this will benefit our patients.

So the vision remains the same. Thank you very much. I hope I haven't gotten us too far off time.

(Applause.)