Concepts and Challenges for Developing Personalized Nutritional Medicine

Dr. Jim Kaput for The Critical Path Initiative

MS: Well, hello everybody and welcome to the first in our series of lectures presented by the Office of Critical Path Programs and the Critical Path Steering Committee on Critical Path Grand Rounds. This is a series that we envision taking place every second month. And the aim of the series is to enhance the scientific communication between the different centers in FDA. We're aware of the fact that there a lot of independent scientific programs that go on within the different centers and this is our attempt to increase the communication in those areas.

As this first of this series of lectures it gives me a lot of pleasure to introduce Jim Kaput. Jim Kaput is from NCTR in Arkansas. He's very kindly come through here representing the Division of Personalized Nutrition. He has a background in biochemistry from the Rockefeller and the University of Illinois. And he's going to be speaking to us about personalized nutrition in medicine. I think this will be of interest to all the centers and I hope this will stimulate a lot of interest in our future lectures in the series.

We would also encourage feedback from the audience as well as input into future lectures that might be added to the series. Without further ado, let me present Jim Kaput. Thank you so much.

JK: Thank you for the invitation to come and speak and I have a fairly difficult task ahead of me. I could tell you specifically what the division is doing and the structure and all of the toys, big pieces of equipment that we have. But what I would rather do is to give you this overview of how do we think we need to go about studying health and biology in order to do personalized health care and that's personalized nutrition in medicine.

Now in the end I'm going to weave in some of our specific projects because we're also looking for collaborations here at the various centers. So you'll see this slide several times; it's sort of the roadmap of what we're going to do. I'm going to give you a brief introduction about nutritional genomics. I'm going to tell you why it's difficult to study. And then I don't like to just complain; I always like to try to come up with some solutions. The solutions that I'm going to propose to you may not be right, and in fact that's why I talk, I'm trying to be an old fashioned scientist to say here's the idea, let's rip it apart and improve it.

And if you walk away from this talk thinking that way it would help a lot because I don't think there's any one person who can really understand how we're going to be able to do personalized health care. And I think we have to tackle it as a team. And then part of that is how are we going to do this personalization using complex teams. So this is a big buzz word in the agency as well as outside in the biomedical community, and you want to know about biomarkers.

Typically, we have biomarkers for disease. My HDL level is high, so they think I'm going to get heart disease later. And if you're a nutritionist and you want to study

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about that, a process for let's say cholesterol you'd say let's just look at the person's diet. So we have a whole set of people, the nutritionists, the nutritional epidemiology that really ignore everything except dietary parts and they say it just depends on what you eat, eat a low fat diet.

Well, I already eat a low fat diet and I try to work out many times. I still have a cholesterol problem. So then the geneticists come in and they say well, this is a genetic problem and all that we need to do understand this is to look at your genetics. So when they do their studies they ignore the fact that we live in an environment and that we eat. And unfortunately this is still true if you go to the latest Genome-wide association studies and you look for the word nutrition or lifestyle you may find it in the article but you won't find any data.

So they're doing the studies as if the DNA that we each have exist in a data bank and all they've got to do is to find the right associations, ignoring the fact that we interact with the outside world. Well, what we now know of course, and this is a subject of numerous studies and reviews in books, etc., which I can give anybody who wants, is that the dietary chemicals interact with biological processes at multiple points from metalation of DNA which is involved in the one carbon pool in folate in vitamins, through transcription factors, which by specific dietary chemicals and RNA turnover and proteins.

All of those processes have been shown to be regulated by dietary chemicals, not just by insulin and not just by some change in the energy content of a cell, but actually by the dietary chemicals. And in reality we've known this for many, many years that what we eat affects our biology and that we're all individual. In 1956 there was a book published by Roger Williams, which is still available on Amazon called Biochemical Individuality. And what he did in 1956, you remember three years after Watson and Crick is he summarized 50 years worth of work that show that each individual human will be in a range. And there's a big range in normal homeostasis. And I'm going to get back to that point later.

Now the reason that we're trying to focus on this, this is like the conceptual backbone of everything that I'm going to talk about today; it's genotype times environment interactions and that's a statistical definition from this person here, a different effect of a genotype on disease in persons with different environmental exposure and at the same time a different affect of an environmental exposure on disease risk in persons with different genotypes. And you don't have one without the other.

So let's go back to how we do the studies. The geneticist says I want the statistical main effect only of the genotype. And the nutritional epidemiologists say I want this statistical main effect of the nutrients, so we're just going to ask what eat. What we need to do is focus on the interaction term; that is the genotype times environment interaction. And I'm going to give you some examples of what we mean by that.

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So let's just take this dietary chemical which is used around the world. It turns out 70% of the world's population is sensitive to this chemical. I shudder to think what would happen if we have this data at the FDA now before the historical fact that we have this chemical in our environment. And what happens to the people who are sensitive they get intestinal bloating, diarrhea, and if they have too much of the chemical they can die from water loss.

And if you look at different populations around the world you'll find that if you come from Europe then you have only a 5% chance of having those symptoms when you drink or eat this chemical. And if you come from Southeast Asia then most of that population is sensitive to this chemical. And what this is lactose in milk. Now we know this, everybody knows this that there is lactose intolerance and yet it really depends upon what your genetic makeup is and what's your exposure. If somebody from Southeast Asia doesn't drink the milk they won't have those symptoms. Likewise I can drink milk and I don't have those symptoms at all, at least not yet, maybe as I get older it might change.

Okay, so how do we study this? We look at the polymorphisms or the variants in the genes. So it turns out that about 10,000 years ago there was a mutation in the gene that encodes the lactase that allowed expression as adults. Remember the reference population here are the Southeast Asians. Most mammals don't drink milk from mom after they wean. So the people who are the reference population are the ones who are lactose intolerant.

But in northern Europe this was a selective advantage because it allowed water, calcium, and protein to be available in the winter, so as an extra food source and therefore it spread through the population and it got fixed and now we only call it a polymorphism. So when we first developed this slide in 2004 we knew this data already, these data, so we said blindly well, the reason we have this gradient is the farther away from Europe you are the less likely that you'll have that genetic background.

Well, that made sense then, but then subsequently somebody did a study in Africa and found that in a sub-Saharan African population they had a different polymorphism which did the same thing. That one arose 7,500 years ago. So now we can no longer say let's just study one population and extrapolate to everybody in the world; we actually have to study each individual population because the biological affect, in this case just lactose intolerance, varies depending upon the specific population you come from.

So that will be lesson number two. So genotype times environment and we have to change genotypes and essentially environments. So that just gives you the overview. And now we can ask questions about why is this difficult to study. Well, you know intuitively why it's difficult, because we have nutritional compositions which vary

depending on where you grow the food, how you process it, how you prepare it, different growing seasons will change. Your culture will change what you eat. So there's a huge complexity in our food environment.

And then health and disease pathways vary. And I'm going to show you a specific example about Type 2 Diabetes down the road. And then also our genetics varies. So what we want to do is to produce good nutritional food. And in a shopping center near where I used to live in Chicago they're asking us in that aisle you can buy nutritional foods next to the Pop Tarts and the other prepared meals.

But this really is what our consumer or our customer for the FDA wants. They want safe food. Yes, that's a very big problem. But also we should be providing food that doesn't make people obese. And you know if we look at it we actually have a bigger problem with chronic diseases caused by our food supply than our food safety problems. Now that's not to denigrate the fact that we have to look at food safety and protect people, but we also as an agency should start re-focusing some of our efforts at least on how do we protect the health of the population by preventing the onset of disease. And I'm going to bring that home later.

So this genetic problem, genetic heterogeneity those two graphs down there are bars and each individual vertical bar with different colors is one individual. And the top are people from Porto Rico and the bottom are people, Mexicans in San Francisco. And what you'll see is even though the geneticist would call all of those people Hispanics, and by the way we would also call that culture a Hispanic culture even though they might be Mexicans in San Francisco and Porto Ricans. You can see the diversity.

So the red bars represent the contribution of native chromosomes, Native American chromosomes to that person's genetic profile. The yellow are the contributions of Europeans to that person's genetic background. And the green are the contributions of African chromosomes. So person number 12 on the top here which you can't really see, but believe me, all Native American chromosomes and yet we call that person Hispanic. And the person all the way on the end here, on the bottom, we call Hispanic they have no Native American chromosomes; it's completely African and European.

So when we're doing genetic studies and we go let's throw all the Hispanics together. And I'm going to show you a slide later that this is also true of Europeans; there's no one group that's not heterogeneous in genetic makeup. We're doing a disservice because we're mixing all of those genetic contributions into one. So for the food composition this is just one example. When I started doing this work somebody came by, an oil chemist from M&M Mars in the early 1990s and I was telling him we were changing corn oil levels because we wanted to look at the fatty acid composition and he said yeah, there's a couple other things in corn oil.

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So I couldn't do that work, but he took our samples and gave us back seven data tables. And I'm only showing you four; sterols, triglycerides, five different types of vitamin E, Tocopherols, all the fatty acids vary. And yet we as nutritionists or the team I was working with who are the true nutritionists, were looking at it just as if the fatty acid levels were changing, not looking at any of the other minor constituents.

Now why would that be important? We were comparing corn versus coconut because we thought we were comparing fatty acid levels. But look at this one compound called beta-sitosterol, which is present at about a half a gram per 100 grams in the corn, but it's much less in the coconut and the soy. So when we were comparing these different oils, thinking that we were just looking at one, we were actually looking at multiple different chemicals.

I highlight this particular one for a very good reason. In Europe they put beta-sitosterol into margarine. That's very good if you have a cholesterol problem because it blocks the uptake of cholesterol in the intestine; it's a competitor. However, your gut micro fluoro, or at least some gut micro fluoro can convert beta-sitosterol to androsteindion. That's the type of steroid that Alex Rodriguez and Mark Maguire and those guys are taking. But in women, men too, but women would be more important, androsteindion is the immediate precursor to estrodiol. And you may know that a women's lifetime exposure to estrodiol is a risk factor for breast cancer.

So if you have a susceptible genetic makeup and you live in Europe and you're trying to reduce your cholesterol uptake for your heart health you could be looking at some other problem in another system. And so that's another lesson is that we can no longer think about what we do in the laboratory or in the applications as a single target because biology is complex and it's interwoven, all these pathways are interwoven.

So I also wanted to show you about the complexity of a disease state. And we use Type 2 Diabetes because it's a very good example. When we first started this work people could actually understand that genes and nutrition mattered because if you knew somebody who was diabetic the doctor would almost always tell them to try controlling the glucose levels by diet and exercise. Now it turns out when you do that, assuming you do it, only about 15% of people are helped by diet and exercise. There are a lot of reasons for that and we can go about that later.

So let's look at these 85% that require a chemical intervention, a drug intervention. Typically you get one of the first three drugs, sulfanylureas or usually biguanide. But look at the last line here, the last column. Sulfanylureas only work in 50% of the people. Now if Type 2 Diabetes is one disease, tell me, how come it doesn't treat 100% of the people? Now in the FDA we all know about pharmacogenomics, we know there's variation. So this is not terribly surprising for this audience. But I use

this for the non-expert to show them that genetic variation matters and that physiology matters. The reason is in the color code that I have here.

The first two drugs target the pancreas and stimulate insulin secretion. So if you're a diabetic that has problems with insulin secretion then that drug is going to work in you. But you may have another abnormal pathway in glucose production from the liver, gluconeogenesis. And biguanide acts on the liver in that pathway. So you might be the type of Type 2 Diabetic that has a problem because you have too much glucose production, not necessarily because your insulin secretion is deregulated.

And then there's alpha-glucosidase inhibitors which are in the intestine and then TZDs or PPA argama, analogs that target peripheral tissues. So when a diabetic walks into the room, into the clinic we don't know which of these pathways is apparent. In addition, there's genetic variation within the pathways. So on top of the physiology that is disrupted, which of course has an underlying genetic and nutritional component, we also have genetic variation now.

So it's no wonder that if you talk to somebody who works with diabetics, and I've done this a number of times, they want to know how do we put people into different groups so that we know how to treat them. That's one of the reasons we have pharmacogenomics. So here's another problem though is that we have a range of genetic susceptibilities. And I'll show you why we have that range in a minute. And we also have aging that occurs and changes how our genetic profile interacts with the environment.

So if you're genetically lucky and you're at the bottom end of the genetic range for Type 2 Diabetes, then you probably will not be affected much by the environment, you might be the 5% lucky people who can have ice cream and fatty foods every day and not have a problem. But most of us have different susceptibilities, depending on how we're born. And that ribbon is supposed to show you what happens when the environment interacts with our genetic makeup.

And then finally one group of people who have a genetic predisposition to the disease; it doesn't matter what environment they're in they will get it. And we actually know that that occurs. The problem we have is that when you go to the physician you're somewhere in this range and we have this artificial way of where are you right now and we're going to project out based on the symptoms that you have to your outcome.

We don't know the starting place in the genetic, that your potential is, we don't know what the gene environment interactions are that affect you, and we can't predict the outcome really except for some clinical markers that are rather imprecise. Now we do know that if we can change environments we can change the outcome. So that's part of what we're trying to do; design genetic tests and environmental changes that can influence disease outcome.

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Now I said it was complex. The Genome-wide association studies also confirm that. Here are the ten latest genes that have been shown to be involved in Type 2 Diabetes. There are about 20. But these are the ones that came through the Genome-wide Association. And if you go through this list, which we're not going to do, they are in different cells, different pathways, just as predicted by the drug responsiveness.

Now there's a lot of problems with the GWA studies and after the seminar you can listen to me harangue most of that. The biggest problem is they didn't do any environment at all. But in addition if you look at the total contribution of those genes it can only explain about 2% of the total disease. So there are either more genes or the environment matters and we have to start looking at those genotype times environment interactions. And as you've heard earlier all of these studies were done in Europeans. So if you happen to be a non-European these may or may not apply to you; we don't know.

So I want to go through one aspect when we're talking about pharmacogenomics and nutrigenomics and let's just say that there are these six individuals, A through F and there are these ten genes, and let's just assume for the sake of a statistical argument that there's only a protective allele, if you get it it's helpful, there's a nutria allele it doesn't matter what your environment is, and then there's an allele that's negative; either the environment causes a problem or just genetically it's going to cause a problem.

And let's just distribute these among all the people, this Individual A, is the genetically lucky person, Individual F is the genetically unlucky, most of us are in between. How many of these combinations would we have to know in order to do a genetic test, in order to say what our risk factor is? So within one individual there are 59,000 combinations. Now the geneticists in the room or watching will know that there are not 59,000 combinations, because of allele frequencies and genetic histories and blah, blah, blah.

But there are still going to be lot; there are going to be about 10,000 or so combinations. And yet we have companies out today, deCODE Genetics is one, in which they will test only PPA argama and tell you whether or not you have a risk of getting diabetes that's 1.25 fold greater. So, quite frankly, it can't be done, because look from even a Genome-wide Association studies and just with three alleles and there are probably more, there are lots of combinations that we're going to have to test. We're not even close to this at this point.

So in addition to that complexity we also have this complexity, which goes back to this genetic ancestry, which is they look at a set of four polymorphisms, which they call a haplotype, a set of gene variations in a short segment of DNA called the haplotype K. And they looked in European Americans and they looked in African

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Americans. And you can see if that if you inherit that haplotype K from Europe your risk of heart disease goes up 1.3. But if you're African American that population risk goes up to five.

Now there are other explanations for these data, but it does say that the effect of the genetic change depends on your genetic background. So we need to put in our studies people with different genetic backgrounds. Now I'm going to use an analogy here, because I'm a biochemist by training, when we're in a laboratory we didn't just isolate a unit of enzyme activity, you know one day to the next, we looked at the unit of enzyme activity per milligram of protein.

And yet we geneticists are going around telling people you've got that snip you're going to get diabetes. Well, maybe, maybe not, because the rest of your genetic background may change the affect of that genetic marker that you're testing. So what I'm saying that we need to develop is snip per genetic background. And oh, by the way, I think this is true in our clinics too.

My cholesterol level of 230 may give me a heart attack in five or ten years. I'm taking an 8% chance. But it also could be perfectly natural for the rest of my genetic makeup in that the rest of the genes that I inherited from my mom and dad can handle that 230 and I would be perfectly healthy. We do know people who drop dead of a heart attack who have cholesterol levels under 200. And likewise we know people last until 100 when they have cholesterol ratings that are much higher. On average it's not good to have high cholesterol, but personally it may be okay.

Now how do we do this genomic makeup? I have no clue. But we're going to start trying it in our laboratory by testing everybody's genetic ancestry and then we're going to hand the data over to some wonderful statisticians that I work with at NCTR and we're going to see if we can construct the number that reflects our genomic makeup. And if we can do that then when we say that you have a snip for Type 2 Diabetes we'll know whether or not it's going to affect you because we'll have some measure of your total genetic background. And I think we have an approach to do that.

So that gives you the background of why this is a difficult field to work in. So, if my job is to be the Director of this Division of Personalized Nutrition and Medicine, and what a cool title, how do you do it? Do you do it the way that we've been doing it or do you think about a different approach? So how do we do science right now? The way that we do science right now is we take a population and we look at the controls and then we pick some cases and those are diabetic, obese people, cardiovascular people and we say what's the difference?

And what we find is that there is some risk factor, that's that population-attributable risk. But think about it, it tells me that population control is, on average, different than population case. That doesn't say anything about me; I wasn't in that study and

most of you probably were not either, not only your genetics, but also your lifestyle. So if that's the case, how are we going to do our studies?

So here's a pictorial representation of what I'm talking about. We characterize people, cases and controls and then we just put them into these different measuring weigh boats and we say tell me the difference between the people in the first group from the second group? And we come up with some average phenotype. And lately what we've been doing is we've been asking what genetic make up you have, and therefore like the first row, genotype A is present a little bit more in the cases than in the controls, but if you just looked at the first one, like one snip you would get an answer.

But look at the last one, Snip Set C, let's call it that where it might be a protective affect. It's not a negative allele, but a protective. But it wasn't in the study so we never picked that up. So depending on what the lamp is that you're looking at you can come up and say well, we know genotype A contributes to disease and we're done. Well, that's what we've been doing. The question is, how are we going to do it better to get personalization?

So, the concept that has been very rampant in the field of genetics, and to some extent nutritional epidemiology, is they've said we've done our previous studies with 1,000 people. And we know that the signal was very small relative to the noise. So let's now make it 20,000 people or 30,000 and a lot of times in the American press you'll hear we're going to do 100,000. What's the assumption that they're making there? The assumption that they're making is that all the people who are added to the study are genetically just the pool that they started with; everybody; the same, genetically, culturally, everything.

Well, think about that, right? So if I have this half of the room is the original population and now I have this half of the room, it's a different set of people that you're adding in. So in reality when they think that by increasing the population size they're raising the signal, they could be increasing the noise. So there's a sign in Bill Slikker's office, my boss, which I picked out the first time I visited him when I went for the job interview, and it's by Albert Einstein, we keep doing this experiment over and over and we expect to have different results.

Well, you see Genome-wide Association studies and you're still giving them lots of money to do it when they're not looking at environment as part of it. Now I've been told that they're starting to put environment in, but they're still putting [in] lots of people and putting them into the same pool. So I don't think the results are going to give us much more.

And we know this to be true because even from the Genome-Wide Association studies this is a map of Europe in which they did an analysis and they can put these, or each individuals, each of these little dots are individuals, and you can see they

cluster so that the people in Portugal and Iberia cluster, the Italians cluster, the people in Switzerland cluster, etc. In fact, this paper in 2008 said that with this data, these data you can place anybody from Europe at a 50% accuracy to within 200 miles of their origin of birth or their IOP grandparents origin of birth and 90% within 450. My ancestors are from Poland. If we did this study my ancestors should be somewhere up in this area over here.

So the Genome-Wide Association studies in Europe dumped all these people into that pot. Look at the difference between us. So there's a very big difference. We can't predict what it is but we've got to start thinking about doing the experiment in a different way. So here's what I'm suggesting. And this is where you can start throwing the tomatoes at me if you want. We haven't really analyzed the total genetic variation in the world or the total phenotypic variation for literally any phenotype, except maybe skin color.

You can line us all up, from the darkest dark to the albinos and we can look at that and for the most part it's going to be a gradation where you can't get groups. But in reality we group people, a statistician can group people by skin reflectivity or whatever, we can probably create groups. Height would be the same, maybe weight. So the idea would be hair, if you took one group that's phenotypically different from another at the extremes of the phenotype and asked what's the genetic difference? Now this isn't my idea, I wish it were.

Mary Claire King did this in the early 1990s. She put women who got breast cancer before the age of 35 in one of these bins and women who got breast cancer post menopausally in the other, and she found the breast cancer locus. All I'm saying is that we've got to do this with metabolism, especially for obesity and diabetes and cardiovascular disease.

So we first find the different phenotypes and then we compare the extremes and then using good statistics we can fill in. Now it won't be clean bins throughout, but we'll still get clusters of people, at least that's the prediction. And ideally you don't want to do this with a phenotype, you want to do this with genotype times environment interactions; the statistical main affect for how we live, because it's not just the genes that I have but how I interact with the environment. And then after that you can compare genotypes and phenotypes in various things and you can fill in the bins.

So here are pictorially is how it would represent. You can actually do this in any sample size. In fact, we have some studies that are relatively small groups. You first characterize phenotypically and genetically as much as you possibly can, get as much data sets, genomics, proteomics, metabalomics and phenotypes. And then from that you can say let's reduce the dimensionality of all of those different data sets into something that makes sense, and we have algorithms that can do that, and then you'd use classification algorithms and you put people in the bins.

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Then you'd go okay, let me test that, this is just the training set. Now let's go to a larger population, characterize them with the same tools or perhaps a refined set of tools and then we can then sort them into these bins. So there's an approach here that we don't first say you're different from this person, but you characterize everybody first and then you ask how they sort. And we can do this. We have the algorithms and we have the tool sets now to do this. What we typically don't do though is that if you're a geneticist you only do genetics and you don't do anything else.

If you're a nutritionist you look at that. If you're a metabolonic person you don't do either of the two. We've got to put all of these different sets together in order to get these groups. And again don't think that I'm saying that we're all going to be in these metabolic groups and going to be completely clean. There's going to be some spillover. But if we can get most people into the metabolic groups I think we can design better diets and nutritional advice.

So that's the proposed path. And I would ask you to think about it and send me e-mails, nasty or not so we can try to improve that. Now, okay, so that gives you sort of the overview and in the next ten minutes I'm going to tell you what are we doing tomorrow, this is all nice on the slides, but we've got to start converting it. So first of all lots look at what do you need for personalized nutrition in medicine? You need genetic and genomic analysis in the future. I don't think we're going to get by saying we're not going to have it. Our genes for most of us don't change during life unless we get some mutation.

We might have different methylation patterns but for the most part what mom and dad give you is what you start out with. So we're going to have to do genetic analysis in the future. We need evidence-based data and mechanisms. It's not good enough to say here's your diet, you know, your blueberry extract, it's an anti-oxidant it's going to make you feel better and really be able to tell people how many blueberries to eat. You've got to know why it works.

We have to have good health and disease biomarkers. And let me just go back to this. Think about this with that aging graph I showed with the complexity of the Type 2 Diabetes, with the genetic complexity when we do our biomarker analysis we really don't put all those variables in. So one biomarker might be good for somebody who's 20, it might not be good for somebody who's 50, because the aging process has changed the level of the expression of the gene or the protein.

And yet we produce biomarkers as if they're good for everything without knowing all of the various components. We know we need nutrient and activity assessments. And I must say this is the biggest problem we have experimentally. I can do your genetics, no problem. It's very trivial actually to do that. But tell me what you ate

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last Thursday for lunch and I don't think you'll be able to do it. And even if you did I actually probably could tell you because of the way I eat.

I couldn't tell you how big that apple was or how big the banana was, right? I could tell you how big the yogurt cup was, but that's about it. But if you ask me for dinner I would know that too probably but I wouldn't know the amount. So we have to come up as a field of nutrition and nutrigenomics of how do you do better nutritional assessments. We're actually going to have a meeting here in Beltsville with the USDA, the FDA, NIH and hopefully CDC plus some outside experts to talk about what is available right now for nutritional assessments and physical activity, what do we need and how do we go forward.

So we're going to try to address these issues soon. And then I also have a strong belief that it's very good for us to develop academic results in my laboratory, but if we don't think about how we're going to implement that for people then I don't think we're doing a full job. So in all of our studies, particularly those that we have in communities, we do a cost/benefit analysis, or at least we're going to start, so that down the road when you say well, that's fine and dandy you did it over in this lab, how much is it going to cost over here in Washington to implement that part of the health care problem or access to that health care I might have a number at least for my components that I work with.

So the Division of Personalized Nutrition in Medicine has created a tool set or has a tool set. We have a limited amount of metabolomics, but many other people at NCTR have the proteomic machines and the metabolomic machines. We have a very good biostatistician group led by Jim Chen. We have a genomics core that we developed that will be able to do medium throughput genetic analysis, DNA re-sequencing. We have an alumina b station (ph.) for genomic analysis. And in the typical small sets of equipment that most people have we hope to get an Nex Gen sequencer down the road because, as I said, we're going to have to re-sequence genes. And I'm going to show you that in a minute.

So that's like our toolkit. And then we have some model systems that we're working on. We're developing a stem cell program. Two people in the division are doing that. We have animal work that we're doing, model systems. And then we're going to have some human studies as well. So the human studies, there are two different ones that we're focusing on. The first one is based on an idea called community-based participatory research. Some of you are shaking your heads and that's great.

The idea here is that if we did the epidemiology studies like before I would come into this room, I'd ask you each to roll up your sleeves, I'd take the blood, I might do nutritional assessments and then I'd walk out and you'd never see me again. I might publish the results, but it's all anonymized and you wouldn't know what it is. Community-based participatory research doesn't do it that way. We go into a

community, we form relationships with people, we ask them about their lifestyle, we ask them to participate.

They might tell us what medical problems or what biomedical problems they would like to see analyzed in their culture. And then we work with them; we ask them about changing their lifestyle, we encourage it. And maybe six months later they will, we'll go back in and reanalyze it. And this is an approach to research which is normally used in the social fields and has started to be used in the nutrition community. It has not been used in the biomedical research field, except as I'm aware of an Eskimo population that's looking just at genetics, nothing else.

So what we're doing is we're doing this in a region of Arkansas, which I'll show you. And it's very idealistic and I'm not shy to say this; we're looking at each individual, but they live in a family and they live in a community and if we're going to change our health care process, yeah, we can have policies up here in Washington to do it, but we also have to start it from the bottom up. So we're working with each individual. And the way we started this is that the USDA had an 11-year program in community-based participatory research in the Delta region of Arkansas.

So for those of you who are politicians this was one of these earmarks, although it was an earmark that was trying to improve the nutritional quality of the people who live in this region of our country. This is perhaps the worst health statistics in the whole country, even Appalachia doesn't have these as badly. We're doing a project in children. Forty percent of the girls in middle school in Marvel, Arkansas were obese or at risk for obesity, 40% of the girls. And the boys are just as bad.

So what we're doing is we had an obesity-prevention summer camp that was developed by the USDA run at a community center that ran a summer camp for 25 years by a woman by the name of Beatrice Shelby. And what we did is went in last year and said we would like to do biomedical research, let's take your blood and do vitamin analysis. And the idea last year was very trivial. It's a very simple experiment and you'll actually laugh probably because all we did was we said what are your vitamin levels before camp starts, what are the vitamin levels after we gave them two meals per day and a healthy snack, after five weeks what changed, and then one month after they returned home.

Now obviously for those of us you would say why even bother. But in reality this group does not have access to healthy foods. And I have pictures of their stores. The closest Wal-Mart is about 20 miles away. Many people don't have the money to travel that far, so they usually buy their food at the gas station and usually it's greasy food. So the trivial idea is that in the kids at the summer camp the vitamin levels would go up and when they return home it goes down.

So we tried that last year. The meals weren't designed the right way and we probably won't get much data. We're analyzing the bloods now. But we've gone back

to the community. In fact, the community has asked us to come back and to expand our project. So this year we're going to try 100 kids and we're actually going to try some genetics. And we're going to start looking at genes that are involved in micro nutrient metabolism that map to quantitative trait loci involved in obesity. That was a lot of genetic jargon there, but essentially the pathway genes that overlap obesity locus, right? So we're putting those together.

The reason we're doing that is actually, it's a hypothesis, but it's actually pretty well-founded in that we know maternal nutrition and early childhood nutrition tell you a lot about adult onset disease. In fact, if you don't have good maternal nutrition and good childhood nutrition you have an increased risk of all the chronic diseases we know. So there's got to be a genetic connection with early nutrition and vitamins are an easy one to look at. So that's one. It's 100 people. It's relatively small.
The second one … did you have a question?

Q: [Un-miked question]

MS: The question is, why choose Vitamin D? The reason we chose the different vitamins is what we could analyze on one HPLC column. You're absolutely right in that some of the vitamins that we're trying will not change because of distribution, etc., some of them are going to be higher than others. Although the USDA has done a study of what the children and adults eat, not what's in their blood.

And it's a shocking slide, which I don't have in this group, but they are deficient in zinc, vitamin A, vitamin C, vitamin E, total energy, specific fats they're all deficient in. So we don't think that every single nutrient, we're actually looking at 13 of them right now, we don't think that every one will change, but we're hoping to see some.

So that was one. The second project that we're starting that's been approved in the concept stage is this idea about how do you create a biomarker? What is a biomarker? The one we want to focus on is actually something that we don't even talk about, what's a biomarker of health? So let's say Joe might be healthy, I might be healthy. We might be healthy in two different ways. How do we tell the difference that we're both healthy even though we have different shapes, different ages, different lifestyles, etc.?

Most of the time we measure these biomarkers in the biological state of homeostasis; overnight fast, what your fasting glucose level, what's your HDL level? But we already know from way back in 1956 that different individuals are different. Yeah, we're all normal, we're different, but we're normal. So the idea that we've come up with not only me, but actually a number of other people, is let's perturb homeostasis, let's perturb the resting state and see how you respond.

The prime example of this is something we already use and know, which is the Oral Glucose Tolerance Test. So if you're giving somebody a bowl of subglucose it might

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look about like this and wind up about in this box, they're considered normal. And if they respond a little bit greater to the glucose over time and they wind up in this spot we call them impaired glucose tolerant. And then if they have this response and it stays elevated after two or so hours we call them diabetic.

Now the change we're going to make is we're not only going to do the glucose levels what we're going to do metabolomics in many different systems, like amino acids. Now again this isn't only our idea. They've done this in the TNL in The Netherlands. And what they found for 19 people is that everybody had a different profile and everybody's amino acid levels changed. But they only had 19 and they're all Dutch, so we want to expand this type of idea to see whether or not we could perturb what your resting state is and find whether or not a specific set of metabolites can predict your existing health and also if we follow you longer your susceptibility to disease.

So it's a different way of doing the study. And we would want to measure after this zero metabolites, immune function, oxidative damage and perhaps some urine metabolites, how fast you excrete some of these things. The way we're going to do that is we can do a whole series of challenges. Right now we're thinking of the oral glucose tolerance, we may do a lipid tolerance test, and not just eating a Big Mac, but a Big Mac might work.

An activity challenge, which is an exercise challenge, how do you respond if we put you under some stress? We do that anyway, but we're going to be measuring these metabolites. And when we floated this idea, Federico Goodsaid over here at CDER suggested we do Tylenol as an over the counter drug challenge. And as you know there's liver toxicities that occur with Tylenol. This might be a very good concept to think about how you would test over the counter medications.

And then we would do different doses, kinetics and relevant physiological measures. And remember we don't have a reference population; every individual is going to be their own reference. We're going to do a before and after. So the way we're going to design this experiment is we're going to do a weight intervention and I'll show you that in a minute. So we're going to have a registered dietician talk to us about how people should maintain weight. We're going to do a complete clinical analysis. We might do, we will do dietary assessments.

We also may do heavy water to check on those. We'll do activity levels. Heart rate variation. Carl Sarniglia at the NCTR is a microbiology expert that looks at the gut microbium. We will do that. And then we're going to look at genetic ancestry on everybody. Each individual if they choose will be counseled about how they could lose weight or get in better shape. But then we're going to do this thing called the challenges. And the challenges will also be done in the baseline. They will be things like the glucose tolerance test, lipid, aerobics, over-the-counter drug or mental acuity test.

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Muriel Paul and the neurotox is setting up that. And these are the assays that we will do for each of those. So for the glucose we're going to do C13 flux. If we do the lipids we can also look at that. For almost all of them we'll look for the immune function as a consequence as the challenge. We'll look at selected genes by re-sequencing of those involved in the particular challenge. We'll look at DNA damage, aerobic activity gives you DNA damage. Some of it's good, some of it isn't. And then we can also look at selective proteomics. So then we'll do this intervention.

It will be an individualized intervention for weight management. At the end of the six months we will then redo the challenge test and we'll redo all of the clinical assessments. And we can compare pre and post. How did I respond to doing exercise five days a week instead of three or two and a half or zero, depending on how much I travel. So by this intervention we'll be able to look at how each individual responds. Now granted, we've already done a survey and about 100 people at NCTR will be the lab rats. I'm going to be person number one in the cue.

I think this is going to give us a lot of information about each individual. Remember after we get all the information and then we may try to sort, we'll have only 100. So after we do this year next year I'm going to knock on the door over here on at White Oak and see if we can do it here. I hope that you guys will do it. And in the RD that I'm talking to said six month's intervention is not really very good because anybody can behave themselves for six months. You want to see what happens after a year or so. So we may do another set of something at the end of another year.

So those are the two human studies that we're working on. They're local. They will give us a lot of information, but they're too small to really tell us about what happens in China or India or other places. So several years ago a group of us got together and said we got to work internationally because if we don't start doing this and comparing across populations we're not going to get the answer. So we wrote this paper. There were 89 authors on it. It was a feel-good paper. I recognize it was. But it sort of laid out a road map. And I just got an e-mail a little while ago, a couple of weeks ago, it said it's now a highly cited field, after only four years. So if it's around long enough it's highly cited.

Nonetheless, I think it indicates that there is an interest in this field, because not only did all of these people join it, but now people are citing what we want to do. One of the things we realized is that we needed to do genetic variation. So we wrote this paper and then the man who set up the human variation project, a man by the name of Richard Cotton, at the University of Melbourne contacted me. He was the first geneticist that said let's work together.

And he wants to do essentially the same thing. He wants to look at the variation in genes that cause disease. He's looking primarily at monogenic diseases. But when we got involved with him and a couple of other people like me we said polygenic diseases are important too, we can extend this to genes that are involved in drug

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metabolism. We can extend them this for those people who are from CFSAN to those genes that might interact with dietary supplements. So there's a whole range, there are 20,000 genes we have to devote to.

And he would like to have a collection of variation and this is what's different than the Hap Nap and other projects. And it's phenotype. This is exactly what we want to do. So then he's focusing primarily on those that are causing variation and it's going to be a community activity in that there's no one government that can support this work or our nutrinomic project. So we're going to have to do it distributively.

So he wants to have a public catalogue and database. And quite frankly the databases we have our genetics are better than nutrition, but they're still not ideal. So this is actually a very good thing. So I attended the second planning meeting, which was in Spain in May. And we wrote another one of these big papers saying here's what the Varion project needs to do. And we have merged our efforts. They're going to focus on a lot of the activities that we need and their geneticists, we'll let the geneticists do they're job and we'll try to do our job.

So I've been going around the world for several years talking about a harmonized Type 2 Diabetes protocol. And so far these are the various laboratories and the various countries and regions that have agreed to work on this. The concept is can we come up with one experiment that we all agree to and we replicate that with local funding in each one of these different countries. So that I'm not going to say to my bosses at the FDA give me money so I can send them to Saudi Arabia to do this, I'm going to say to the Saudis here's the protocol, go see if you can get the money.

You can publish on your own, but then if we do it in a harmonized way we can collect our data sets. And then we can really compare genotype times environment across the world. Now I've actually got the protocol for most of the biology and genetics all worked out. What we don't know is how do you assess the nutrition in Saudi Arabia and compare it to Arkansas. So we're actually trying to get that worked out before we go forward.

But next week I will be in Saudi Arabia at their invite because you think we got it bad here, 20% of the Saudis and also the Kuwaitis are Type 2 Diabetic. So they're not only facing the rural population problem of under nutrition, but they're also facing the chronic disease problem that we're facing because of our diets and lifestyle of excess calories that are not nutritious enough. So their economies have to struggle with both ends of the spectrum at really high levels.

Now whether this will work, I don't know. But my view is we've got to take the chance and see if we can get it to work. Otherwise we're not going to be able to go forward and do it. So the concept of these international projects and the work that we're doing at NCTR is yeah, I have to have a project on the ground, close to NCTR

today that I work on every single day, but I got to integrate that into the efforts that are going on in the world.

So we want to recruit, we do it locally, we recruit collaborators in populations. We developed a harmonized protocol. Then we have to ask everybody to secure their regional funding or local funding. We conduct a study and then you can publish locally, but also you can compare datasets. Not in a meta analysis way, but knowing going in that you've done the minimum experiment in each country. I could add more because we got a lot of toys at NCTR. I could probably do a lot more than someone in Bolivia, but at least when I know the data comes from Bolivia I can compare the minimal set to what I do at NCTR. And that's the concept.

So we're doing a Type 2 Diabetes. Last week I was in Vancouver and we had our kickoff meeting for micronutrient genomics, which is at the bottom. We hope to get this working and a harmonized protocol of some sort by September of this year and try it. I've got a lot more help with the micronutrient project because we really believe this is a public health science project that could really change a lot if we can deliver hard evidence about the micronutrient levels that are needed in different populations. So we're very excited about this one. It's much easier to change your vitamin levels than your fat levels in your diet. So I think this is a good one to do.

So this sounds really far fetched. It sounds way over ambitious. But I read a book a couple of years ago called *Making Things Work, Solving Complex Problems in a Complex World* by Yaneer Bar-Yam. He runs an institute called the Complex Systems Institute. And he had many take-home points, but the ones that apply here, if you're going to study a complex system and be successful you have to match the complexity of the system to the complexity of what you're studying.

So if you don't match the complexity, if you try to understand biology by being a geneticist only or study biology by only being a nutritionist, you're not going to succeed, because the system is complex and has interactions. So you have to do that. The problem is I'm not an expert in genetics or nutrition. Actually, I have a Ph.D. in biochemistry. I sort of pick up some of this other stuff.

So what we have to recognize that each of us has a limited level of complexity. I know what I can do and I know what I can't. And then I try to find the holes of what I can't do by getting collaborators. So we have to distribute this complexity of the task among many individuals. So I like this stuff. I think we're going to change the world down the road. But right now if you want to be healthy these are the things that you can do. So I'd be happy to address questions. I know it was fast but I think I covered what I wanted to. I hope that gives you an idea of the field, the specifics and also what we're trying to do by linking up with other people. So thanks for your attention. [Applause]

MS: I think there are a couple of questions.

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MS: I'm sure that you've probably heard of the 1,000 genome project.

MS: Yes, I have.

MS: That just seems like it would go into your program there.

MS: Yes. I know. In fact when we wrote the variome paper it was listed in there. And it's like 1,500 people they're going to do. It's actually more than 1,000. But again they're only looking at genetic variation; they're not linking it to phenotype. So it will play a huge role in telling us the regions of the world in which we have to look for more variation. But I would argue that just like the Hap Map there's going to be a lot of rare polymorphisms that will not be detected by just looking at 1,500 people.

So my view is that in many of the studies we have to do we have to re-sequence. But it's all adding, it's sort of like different layers. But the vari ome project differs from the 1,000 genome in that it's trying to collect phenotypes. The nutrigenomic community also is trying to collect phenotypes. So we need the genetic variation but we're adding to that.

MS: These people though that are volunteers in the 1,000 genome project would you consider them as volunteers for your study as well?

MS: I would welcome them. If I could distribute the cost of the genetics to a different group, believe me, I would, because if you give me a pot of money and I've got to do all the genomics and arrange for the metobolomics and arrange for the … can't do it. That's why the complexity, if we could get more geneticists involved, I will tell you that's been one of our biggest struggles is the first paper in 2005 I sent it to a very well known geneticist, I won't name names, they didn't even respond to the e-mails because their mindset is we will find it through genetics alone.

And sorry the experiment is now done and we know it's only 2%. So they have to put nutrition in. Now whether or not they're going to be willing to admit it, Lander did in a recent science articles at the bottom said we have to remind ourselves that people live in environments. So I think they're waking up. But on the other hand the nutritionists among us, including me, have not given the correct toolset to the geneticists to assess nutrients intakes, to assess physical activity.

They are out there but they're scattered and they're very hard to use and they're very hard to find, which is why we're trying to do this workshop idea. Let's get the workshop and get the tools so that it's not a problem to put both into your experiment. Yes?

FS: [Inaudible question]

MS: The question is for the Type 2 Diabetes what's the status of the various projects? We haven't sent it out. We are just now starting to organize in the Delta region of Arkansas with this. We're going to work with Steve Albane at the University of Arkansas Medical School, plus the community has invited us to work there. We want to get 1,500 newly diagnosed Type 2 diabetics. It's going to take a number of years.

The newly diagnosed is important because as soon as you tell a diabetic that they have diabetes they may change their lifestyle. So if you do a nutritional assessment

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two years later you don't know why they got to be diabetic. So you have to … and that creates a lot of problems, because it's starting from ground zero. So the second part of the question is how are we going to do our snip analysis? We're going to be re-sequencing.

Now I know the cost is greater than a chip, but the chip is selected for 90 people from Aruba, Africa, 90 Europeans, and you saw the diversity in the 90 Europeans, 45 Hon Chinese and 45 Japanese from Tokyo. Those are the 270 people that are on that chip and it doesn't represent the genetic diversity that I think exists. So we're going to take the candidates and re-sequence the axons and the promoters of all the candidate genes.

And right now we only have a 3730 so it's a little bit costly. But if we move into this next generation, assuming we get enough sample flow, because it's very costly to get unless you do that, then we're going to be able to reduce those costs. But almost everybody I talk to in the genetic community agrees that we have to start re-sequencing, including the Chairman of the Scientific Advisory Board who visited us last year, Alan Rose.

We had a little off side discussion, I said you probably won't like to hear this but I don't think we should do the arrays, the chips. And he says no, no, re-sequencing is the way to go. So I've gathered some information from various people and I think the genetic variation is so great we haven't studied enough, we have to do the re-sequencing. And I'm hoping that our core can serve those purposes for the FDA not to build an empire, but why duplicate resources?

We have a limb system to handle laboratory information, the sample flow. But we could think about at the FDA drug metabolism, genes, and the genetic variation is better studied than most, but it's still not widely characterized, the dietary supplements, perhaps some of the things that are done in CBER that require biologicals and we might have variation in the individuals who get the treatments or in how they prepare the biologicals.
There's a huge number of things that we have to be re-sequencing for.

So we would like to create somewhere in the FDA a genomic lab that's capable to do that. We're going to have to do it, we're willing to do it with others, whether there be two sites or one site but we should work together whatever. There's a question here.

MS: [Inaudible question]

MS: How do we do drug information in part of the study. So when you do the questionnaires we believe you have to have a clinical electronic … essentially the electronic medical record should be sucked in to the study, so that we would know what drugs you're on. So when I become sample number one at the NCTR Healthy Challenge they will know all the drugs. I'm going to sign a consent that says if we lose some of that information that's okay. But we will have to put that in.

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> And I'll give you another specific example. You guys didn't sign a HIPA for me, but I have a thyroid problem now, just diagnosed two weeks ago or three weeks ago. So my thyroid level is 18. It's supposed to be between 3 and 5. So I have to take thyroid hormone. That's going to affect a lot of the metabolism in the results. So when I do the study that's going to be in. And that means I may be an outlier that we can't really characterize, but we'll put him over there. But quite frankly most of us will have something that we'll have to contend with so we'll have to get all that data in. Yes.

MS: So if you're binning individuals trying to put these together and you have two individuals whose genomic pattern is extremely similar but the phenotypes are wide, and then you have another set of individuals where the genomics is a little bit further apart, but the phenotype is … How do you reconcile?

MS: Because the binning is not going to be on genotype or phenotype, it's going to be genotype times environment interaction. And we're going to look at the statistical interaction term. So it could be that my genetic makeup is identical to somebody else's, but my lifestyle is such that I'm in a different phenotypic bin.

MS: So would you ever use things like principal component analysis to try to…

MS: Yes, that would be one. But that's a linear analysis. Now the problem that we have in biology is we assume everything is linear and it's not. So there are other algorithms, and I know the names and I couldn't do it if you paid me a million dollars, but isomap, they're called non-linear dimensionality reduction algorithms. There's like 20 of them. And then in addition to the dimensionality reductions there's also the classification trees that Jim Chen is the expert in. So we have the toolset. We just have not used the toolset in the appropriate way.

MS: One last question. Are you going to look at (unint.)?

MS: Well, we do have Bob Helfrick from Martha Moore's division. And essentially the design of this Healthy Challenge is I would like to have oxidative damage done, but I'm not going to design that part of the experiment. I'm asking the genetic toxicologists to say here's what we think we can do with this study.

> So we're designing essentially the intervention part and the overall but each individual assay is going to be done by the local expert. So [unintelligible] length is critical especially for aging. It would be wonderful. But I don't know if we have the capability to do that in Martha's division. If somebody here wants to do that we'll send them some sample. Yes.

MS: [Inaudible question]

MS: So the idea would be how come we also didn't try things like smoking or alcoholism. So we will have questionnaires as part of the surveys, the demographic surveys about never smoking and smoking. We may even have number. And again that means that our 100 sample size on the first one is going to be too small to really get an affective bin, because of all the variables. But we're going to start because I think we have to think about doing the science in a different way.

> Now we're hoping that there's going to be enough good data sets to come out that we can come up here and say okay, White Oak here's what we would like to try to

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see if we can get these bins defined a little more, would you like to do it? Now the USDA actually did a study, an intervention study back in the 80s, NCTR did some type of intervention study. And who's better to do it than a bunch of scientists who understand even if I cheat at least I know I have to write it down or at least we hope that they'll write it down. I had two scoops of ice cream yesterday. Yes.

MS: I think we'll have to conclude there. Thank you very much, Jim that was a superb introduction to the series. (Applause) We are very grateful and I'm sure a lot of people will be following up on this to get more of your expertise distributed in the agency. Thank you.

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