The following is from the talk W. Ian Lipkin gave at the WPI on the 24 June 2011.

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WHITTEMORE: Good afternoon and welcome. I'm Annette Whittemore, President of the Whittemore Peterson Institute, and I'm happy to welcome all of you to Dr. Ian Lipkin's presentation on Microbe Hunting. Dr. Lipkin is the Director of the Center for Infection and Immunity and John Snow Professor of Immunology and professor of Immunology and Pathology at the Mailman School of Public Health at the College of Physicians and Surgeons at Columbia University. A physician scientist, Lipkin is internationally recognized for his work with West Nile Virus and other viruses such as SARS and the discovery of the molecular methods strategy, techniques pioneered in his lab. Many of you who are patients who have Chronic Fatigue Syndrome are also aware that he is coordinating a multi-center study of the relationship between XMRV and Chronic Fatigue Syndrome. Welcome Dr. Lipkin.

LIPKIN: Thanks, Annette, for that generous introduction. I don't know how many people in this audience are scientifically sophisticated and less sophisticated, but my talk is really directed to an audience that really does know a fair amount about medicine in some sense. So, if you have questions at the end, anything specific that I have not touched upon, just let me know and I'll try to address that.

I never quite know... I have so many mics hooked up here, I know that at least one of them is working. Is the one that's hooked to the film working as well? Good. So I'll get started.

This is Pandora. Many of you will remember from Greek mythology that she was told not to open this box. She did so anyway, and there were all these diseases that flew out to plague mankind. She was closing it, and just as she was about to close it, she heard one little plaintive voice, and that plaintive voice was hope. And so this is a very nice way of thinking about hope in terms of infectious diseases, including Chronic Fatigue Syndrome. And the attribution is Waterhouse. And as Frank Ruscetti has said, I've selected one of the more "dressed" versions of this particular depiction. There are others that are more scantily clad. This is not meant to be an X-rated presentation.

Now, we find a lot of infectious agents using molecular methods, and finding an agent doesn't necessarily mean that it can be implicated in a disease. And there are a number of ways in which people try to approach this problem. The classic descriptions of these were done by Loeffler and Koch in the late 1800's in looking at tuberculosis, and they really don't fit but just to review them briefly. What Koch said is that you have to be able to find a microbe in every case of disease... and that it had to be specific for that disease. So, for example, let's say you find a herpes virus, which is present in five people and only one of them has disease. It would be very difficult to evoke Koch's postulates, so there are clearly problems with that, and I'll come back to that in a moment. You have to be able to isolate the microbe, grow it in a laboratory, and put it back into the individual animal or human and demonstrate that it causes the disease. So by these sorts of criteria, we haven't proven that HIV causes AIDS, because nobody has deliberately put HIV into humans, to our knowledge. But clearly, I think most people here would agree, that that's a pretty strong link.

So using that as a paradigm and thinking about the kinds of issues that we need to address in chronic fatigue and other chronic illnesses, we have to consider the fact that there are instances where we can't grow a microbe in the laboratory. We may not have an animal model system with which to test it, particularly if there are subtle signs of disease.

Thomas Rivers, in 1937, at the Rockefeller University, began to talk about ... at that point it was called the Rockefeller Institute--forgive me--adaptive immunity. You look for neutralizing antibodies

as a way of proving relation to the disease. The idea is, if you mount a specific response to a bacterium or to a virus in association with a disease, that's very strong circumstantial evidence for that association. And in the mid-90's Dave Fredricks and Dave Rummand(?) at Stanford, both of them began looking at host environmental factors. Excuse me, looking at molecular markers as a sort of introduction to the PCR, and they said, you know, we have to demonstrate that the agent is present using molecular markers. Maybe we can't grow it but that is sufficient. But even here, there are a number of problems and I think the disease that we're talking about is a classic example of that because not everyone who is exposed will necessarily manifest the disease. There can be a whole series of environmental factors--nutritional, there are genetic factors, and so forth. And there are instances where you may become infected with an agent and not appreciate disease for decades.

Now when I typically give talks like this and I have more time, I give a number of examples. But let's just take a couple.

So, botulism. You can have clustered botulinum growing in your intestinal tract or in your skin, but have paralysis. How do you make the link between those two things--something in the GI tract or something in the skin and paralysis? But we have made those links, and as a result, we've been able to find ways in which we can address those problems. Tetanus is a similar sort of an example.

So what we have tried to do is to use a sort of very practical approach. In the instances where we can take something all the way through Koch's postulates: grow the virus, grow the bacterium, put it into an animal, replicate disease, that's the ideal. But there could be situations where we can't achieve that. So we have different levels of certainty in linking the presence of a microbe, or a factor--like cigarette smoking to lung cancer, for example--to the outcome. And the level of certainty will fluctuate at various points in the time that you are finding it.

So, let's say, as a for instance, you find an agent that you determine is associated with Chronic Fatigue Syndrome. Maybe it's XMRV, maybe it's MLV. It starts at the level of being a canidate. Then you need to slowly address, using all these other tools, is there an immunological response to it, can I develop an animal model, can I get some sort of a drug that's specific for that agent and prevent disease? And you slowly build the case until you can convict that particular bug. And that's really what you need to do ultimately, is to convict the bug.

Now this is ... I don't have time to go through all of this, but this paper lays down all of my thoughts on how you go through this process, and it's available on the web.

One of the questions that people frequently ask is how many viruses are yet to be discovered, and the answer is, it's an enormous number. So if you just consider that we've got 50 thousand vertebrate species--talk about fish all the way up to birds and the higher mammals and primates and stuff, and if each one has only 20 endemic viruses, that means there are a million viruses yet to be discovered. That doesn't mean these will all be associated with disease, but it means that there is an enormous amount of work yet to be done. If you actually consider the biomass of the globe, viruses comprise the majority. Because if you look in the oceans, they infect plankton and all sorts of other organisms. It's a massive...massive...amount of viruses. There is a lot of work to be done in virology.

Now, this is just an effort to try to understand the number of viruses that have been discovered, and what factors might lead to their discovery. So the viral sequence database, this is at the National Center for Biotechnical information, has been growing exponentially. The most important factors appear to be improvements in the technology. And there are another couple of things which also come up. The West Nile, people have been looking for West Nile variants or SARS or influenza or endemic influenza and so forth. But really the key is the technology. We started looking at the genome, it was a dollars per genome, now it's down to fifty thousand. I can do it for ten thousand. The numbers are going to drop further. And as these databases become more and more complex, the opportunities to find agents and to look for further association with disease is going to also increase. But again, finding something is not tantamount to proving that it causes disease.

Now this is a New Yorker's view of the world. It's just to sort of vulnerability that we all have. You could do the same experiment in LAX or SFO or wherever else you want to look. From JFK, so this doesn't even include Newark, we have 72 countries direct flights, 190 thousand international flights, 21 million passengers. You can see all of the destinations you can reach via nonstop flight, just from JFK. So, in a one-hour timespan, the green flights are coming into JFK and the red are leaving JFK. See, there is a staggering amount of new material that's coming in all the time.

So there are new viruses and new bacteria being introduced. Everybody's talking now about e.coli but this is just the tip of the iceberg.

The other issue for us is that we are beginning to have industrialized food production on a level we have never before experienced, and pork and beef and poultry are moving all over the world more and more and more rapidly. This is one of the reasons that I've become a vegetarian.

AUDIENCE MEMBER: I thought you liked fish.

LIPKIN: Yeah, well, I don't get them out of farms.

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So, and that's just the legal trades. We have the bush meat trade. It's staggering. In Central Africa, 80 percent of all household protein is bush meat. And you can see these numbers in euros. It's a staggering figure.

Now, we don't really know what's happening in China, but we know it's enormous. This is what's happening in South America. And here, of course, we have Africa, where there is an astonishing amount. Now where do these things typically come through? US borders--there's a lot, but there's much, much more coming through Paris. And we have a big effort to try to understand what's coming in through bush meat in the United States, but they're not doing anything. And of course, we don't really know what's happening in Oceania as well. And the things that people bring in are just remarkable. So we've been looking at risk to the population of the United States from bush meat. You can appreciate that this is a primate head here. This is another primate head, and so forth. So, as we continue to bring these things in, what we find is that there are more and more foamy viruses, retroviruses, herpes viruses. It's an enormous problem.

Now this is from SARS. This is Chen Zhu, who is the Minister of Health of China, and I include this because it shows you how rapidly the world can change. Just prior to the onset of SARS, the Hong Kong government had this travel brochure made up that said "Hong Kong will take your breath away." And within weeks, we had these huge problems. And this is sort of dealing with work on the SARS outbreak. So, again, you go back here and you sort of consider the vulnerability that we have. It's really staggering, okay?

So there are new viruses, plenty of opportunity to find things to link them to disease.

This is from the time of West Nile Virus.

(Laughter from audience)

And this is King Tut in bed with a headache, and this is a student virologist saying, "You know, we're pretty sure it's West Nile Virus."

The point I'm trying to make here is that the most important single person in defining how we operate is not the laboratory person, it's the clinician. Because the clinician says, "There's something unusual about this outbreak of encephalitis or pneumonia or diarrhea, and then contacts people who have the tools that they can employ to address the problem.

Now in this instance, on the human side it was Deborah Asnis and on the veterinary side it was Tracey McNamara. Deborah Asnis was an Infectious Disease Physician who was located in Flushing and Tracey McNamara was a Veterinary Pathologist at the Bronx Zoo. We had crows that were dying in New York, and there were people who were very, very sick. They were typically very active elderly people in their 60's, 70's and 80's who were going outside. You know, they had a lot of motor weakness instead of just confusion and the usual sort of things that are associated with encephalitis. Now, Tracey McNamara tried to interest the CDC in the fact that she had birds dying and elephants and rhinos and so forth, and there was no one paying any attention. So this particular episode more than any other, was responsible for this concept of bringing together one health, which is that humans and animals all live together. We have this communication in viruses, and most emerging infectious diseases start in animals and move to people. So Lyme Disease, HIV, SARS and so forth. The list goes on and on.

Now although we primarily focus these days, and I will in this talk, on molecular methods to discovery, it's key to recognize that culturing is still important. And I know that Judy Micovits does a lot of work in culture, because if you can amplify something in culture, everything else falls into place.

So with SARS as an example, when it was clear from Malik Peiris' work that you can grow SARS in a particular type of cell in very, very high concentrations, everything else fell into place. You could do electron microscopy. You could use a variety of molecular methods, you could use Coronavirus PCR. You could use a paramyxovirus or yellow fever virus primers. There was so much material present, it was so clean, that it was found using any of these sorts of approaches.

So our strategy for looking at unknowns, for doing pathogens, recovering is to take a sample, try to culture when we can. If we can't succeed there, we put it into animals, typically neonatal mice or animals that are knocked out vis-a-vis their immune system. We use multiplex PCR systems that are cheap and rapid. We do arrays, we do hydrogen sequencing and so forth, and this generates candidates that we then try to knock down using a variety of experimental methods.

This is a simplified version of what we do, and it gives you some sense as to the cost: multiplex systems, PCR, microarrays, high throughput sequencing. Again, all this generates for you is candidates, but you then need to prove the relationship to disease.

I mentioned this work that was done by Thomas Rivers in the 1930's. It hasn't really changed that much. We still look for evidence of an adaptive immune response. Has the immune system recognized a new agent and tried to address it? Pathology: can we look at the distribution of the agent in a tissue that's affected? We do challenge experiments in animals and reintroduce the disease. But the problem is, we may not have an animal model with which to do this work. The pathology may be indirect. Sometimes it's not the agent itself. Annette was asking me a little bit about Dengue Virus a couple of hours ago. Sometimes your first exposure to an agent doesn't result in an inflammatory response causing death and so forth, and subsequent ones do. So all of the responses that we see of the host have to be taken in the context of the experience of that individual over a lifetime, as well as the genetic blueprint, in which all of these things are placed as a foundation.

This is a simple method called Mass Tag PCR. It isn't that important. It simply illustrates that what we do is, we take primers, we tag them with things that weigh different sorts of masses, we do an amplification, and we know the size of a particular tag corresponds to a primer set for a specific infectious agent. And then we can rapidly and inexpensively do this work. This was developed by my colleague and friend Thomas Briese. So we have used this over a matter of years now and in a number of different contexts. It really is our first line for detection of novel agents.

The first example I'll show you is one where we discovered a novel virus, Rhinovirus C, which it turns out now turns out to be a very important cause of otitis media, pneumonia, exacerbations of asthma, COPD, and so forth. But we got into this because in this particular year, there was a lot of illness that looked like influenza but was not influenza. So we resolved a third of the unknowns that had not been identified in Wadsworth Center. The New York Department of Health has a superlative state

department of health, I know you have a representative of your state department of health here, and he'll probably agree that it's on a par with any. Is that about right?

(Someone in the audience spoke, unintelligible)

Yeah. Dr. Brown. He's nodding yes.

So, you know, they'ave got all sorts of tools at their disposal. So, the other thing that was interesting was that we found a lot of primary or secondary bacterial infections that were also important, that might have had clinical manifestation.

So there are a number of Mass Tag panels that can be used now. This is $15 to run all these simultaneously, so it's really quite remarkable. You know, some of them are developed by the Department of Defense, but some of them may be of use to you, and you can mix and match. It's like a Chinese menu.

Now this is an example of what we found with Rhinovirus. Remember, this is something that hadn't been known prior to that point. We subsequently determined that from the number that's distributed throughout the world, it's been with us for a very, very long time. Now how would we use this to direct clinical care?

In the work associated with the recent pandemic H1N1 influenza outbreak, we decided to look at what was being called flu. That was flu but wasn't. So over a period of a year, we tracked everything that came in labeled "flu." Now you can see, there's a lot of influenza in June and July, but then it drops dramatically. It comes up a little bit in the Fall, but then it drops again. And coincident with this trough for the influenza, you see this big spike in enteroviruses and rhinoviruses. So if, in fact, you want to treat somebody appropriately, oseltamivir which is pretty good stuff for most viruses. It doesn't do anything for this virus, but we have another drug called ___conerol that will be useful. But as you begin to think about clinical management of infectious diseases, and you have the capacity to do multiplex testing, this will change the way we practice medicine. And it will, presumably, reduce morbidity and mortality and so forth.

Now we did some work, close to here, this was with the Navajo and White Mountain Apache children, and we looked at the appearance over time course to see what it would look like over time to see what it would look like in a rural area, and again you can see here that there's this big spike of Rhinovirus C. Again, this sort of correlates with what we see in the New York metropolitan area.

Now the government of Argentina asked us to become involved in looking at the epidemiology of H1N1. As many will remember, when this was first reported in the middle of 2009, it had a very low case mortality rate, less than one percent of people who were infected with this virus died, which is fairly similar, actually it may be a little bit lower, than traditional seasonal influenza. We lose 35 or 40 thousand people every year to influenza. But instead of 0.6 percent in Argentina, we found that it was much, much higher--almost tenfold higher. So the question was why. Was the virus mutating? Why? So we looked at samples representing mild and severe cases, with sequencing using traditional methods as well as pyrosequencing, analyzed those and found no differences. So those viruses were not evolving differently. What was different became the question. We had yet to look at Mass Tag PCR. What we found was that the individuals who died or wound up hospitalized had a very high rate of strep pneumonia co-infection. In fact the odds ratio was 125. So that means that if you have influenza and you have strep, garden variety streptococcus, you have 125-fold increased risk of having severe disease, implying that perhaps we should be considering--rather than focusing only on an H1N1 vaccine , on pneumovax other streptococcal pneumonia vaccines.

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So these are important things that we can learn from multiplex technologies.

Now, pyrosequencing I primarily... will be primarily what I'm going to talk about today, but the principles are really generic and they are familiar to many of you. What you generate is a series of sequences, and youlook at them at the nucleotide level, and you try to make predictions about what the amino acid string might look like. And then you analyze those bio lines, which means you take those sequences and pull sequences out of a database. You look for similarities between them. And then that gives you an entire view of the tree of life--you have viruses, bacteria, host material, everything that might be present.

One of the weaknesses of the system, however, is that because it's an unbiased amplification system, for the most part, most of what you are going to see is going to be host. So if you're looking at human extracts, tissue, what have you, the vast majority of what you're going to see is going to be human sequencing, not viral sequencing. So you need to find ways in which you can purify and enrich for the microbial sequencing the bacterilum the virus, the fungal sequencing as opposed to the human sequencing or whatever it is you're using in the background.

So this is an example of this in some early work we did. This is actually the first report where people used this approach for looking at human materials. These were three women who received transplants--two received kidneys, one received a liver from an individual who died of a stroke. He had been traveling in Yugoslavia, what was formerly Yugoslavian, a few days earlier... weeks earlier...he returned to Australia, he became sick. He was evaluated there, and found to be brain dead. He had no evidence of any sort of infectious agent at that point, and these organs were then transplanted into these three unfortunate women, who died within a month of receiving the transplants. So they were worked up on a number of locations at the CDC in Australia and so forth without much success. We took them through high throughput sequencing and we found 14 sequences, less than 0.01 percent of the total, and that was sufficient to implicant a novel infectious agent. It turned out to be an LCMV-like virus.

So here, in order to prove the link to disease, we tried to go through whatever we can, so we developed an antibody against a protein made by the virus, and you can see the yellow--excuse me, the reddish pigment which illustrates the protein associated with the virus in the liver, in the kidney of the recipient, and we trans-infected cells with the particular protein made by the virus and we were able to demonstrate that the donor serum was IGM-positive. That means he was still acutely infected and the recipients sera converted. So we had very good, albeit circumstantial, evidence to suggest that this was the causative agent. So there was opportunity to demonstrate the localization of tissue, to show the serum conversion indicating that this--the opportunity because obviously the donor serum still contained the agent and so forth.

Now sometimes we use these... we're very happy to be able to apply these in real time and save people as opposed to looking in post-mortem tissues. This is an example of this. So, a couple of years ago, working with the World Health Organization, we were asked to investigate an outbreak of severe hemorrhagic fever. To our knowledge, it's the most aggressive hemorrhagic fever yet described. There was a travel agent in Zambia who cut herself on her leg. She became sick within a few days and she was airlifted, near comatose, from Zambia to South Africa where there were better medical facilities. Enroute she infected a paramedic and a nurse who received her at the other end. The room in which these three people were kept was cleaned by this woman. She also became sick. All four of these people died. So, even if you consider smallpox maybe 60 percent mortality, ebola rarely gets much beyond that. This last individual, however, we made a diagnosis of the agent. She received effective antiviral therapy, and she alone survived. So, in fact, this is probably the most potent infectious agent yet discovered because it would have had a hundred percent mortality. As it is, it had eighty percent mortality.

So the approach we take is illustrated here. We take these materials through high frequency sequencing, generate the cell spreadsheet, and examine all the different fila within them. You look at fungi in the first column, you look at bacteria on the next page, and then finally, when you get to viruses, you can see quite clearly now there's a whole series of arenaviruses.

Now, as we did this work, simultaneously, in Atlanta and in South Africa, people were trying to clone this agent independently. So the three fragments which were obtained using classical methods are illustrated by these blue rectangles, and the black ones show what came out of this other approach. Well, based on this, we were able to identify this virus as a novel arenavirus. Here it is moving from Zambia to South Africa. It's called Lujo Virus after Osaka of Johannesbburg, South Africa. It is the missing link between the Old World and the New World arenaviruses, and the last individual was treated with Ribavirin as a result of recognition of the agent, and she alone survived. So this was a very rewarding sort of an experience.

Here's another example. I feel badly about this one because, you know, we found it but there wasn't any drug to use. This was a boy 17 years old who had XL-8 deficiency, so he wasn't making antibodies. He was receiving IVIG, and he developed a progressive brain disorder. He was worked up at the CDC in Atlanta and also at Children's Hospital at the University of Washington without success. Two sequences came out of high throughput sequencing. We worked with an Assistant Professor in the group that allowed us to identify this, in fact, as a novel astrovirus, but because the child doesn't make antibodies, we can't demonstrate your conversion, so what can we do? Well, we can still make antibodies to this virus. We can demonstrate it by these pictures. So here we see the frame, and these star-like shapes here are known as astrocytes. They are supporting cells in the Central Nervous System. You can see they light up like a Christmas tree. So these astrocytes are infected. Now the question, then, is what do we see in the rest of the brain. This is a section--these are all sections--of the brain of this child. You can see accumulations of T-cells. You can see this is a neuron fiber. There should be at least ten of them. Going across this panel, we don't see them at all. So what has happened here is this child was exposed, presumably because he lived very close to a mink farm, near Puget Sound, could not make an appropriate immune response. It started in his gastrointestinal tract, went up to his Central Nervous System and ultimately resulted in his death. This is an example of some of the problems.

Now, frequently when we do these high-throughput sequencing studies, we find sequences that are not in a database. What do you do with those? How do you make sense of those?

So this is an example. So this is a library. He's in this library, and there's a big wind that comes through this library and it blows pages of the books every which way. This poor guy has to figure out which page goes with which book. So he looks at these two, and he looks at these two, and he looks at these two, and he slowly tries to patch it together.

Now, these are real examples. The first one, the numbers come from a famous short story by Sir Edgar Allen Poe, that was inspired by the discovery of the Rosetta Stone. Everybody knows what the Rosetta Stone is, right? It's not the thing in the airport. I mean the real Rosetta Stone. So what he did is, he said, you know, Captain Kidd is an English pirate and the English pirates will be using the English alphabet, and I know the times that a typical letter is used in the alphabet in a passage. What is the typical number of times that a number appears in that passage? So he created a key, and he solved the riddle, and he found the buried treasure.

The second one is the Meroitic alphabet.

And the last one is the ___ mission. So this was the actual report which said the ___ battle would continue. And so these are real examples from, you know, history.

Now you can use these in any number of ways. Here's the Declaration of Human Rights. If you just look at the frequency with which two letters are used, or three letters are used, you can create a family tree of words and recognize the similarities between Polish, Czech and Bosnian, Dutch and German, Spanish and Catalan, and so forth. So, this is what we do with sequences when we can't understand in any other way.

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Sometimes when we're looking at the gastroentestinal tract, the question that comes up is, is this a human virus? Is it a virus that was infectingth food? (that the person ate) Is it an insect that was associated with the food? How do you sort these things out. So by using these issues like the frequency with which a nucleotide appears, the order of which nucleotide you're using, you can actually, with fairly good probability, sort out whether it came from insect or a plant or a mammal, and decide whether it's at least infecting a human or not.

Now how would we use this in real life? This is an example that proves that this works ... I should step back for a moment and say, when I started talking with my mother about this, my mother is 87 years old, she was very, very unimpressed. She said ... She said, "You know, during the Second World War, I worked for the War Department as a cryptographer and we solved ... you know, we solved all kinds of things like this. This is really no big deal." So there you go. Anyway, that's what mothers are for, I guess, huh? To take you down a peg or two.

So this is an example ... We were asked by the government of Norway to try to figure out why salmon were dying. Now, salmon are very, very important. You know, they're new...they're harvested all over the world, and these fish were dying with an inflammatory disease of the heart and skeletal muscle. Some of you may or may not know what this should look like, but all these dark blue cells are inflammatory cells that are there in the tissue. But you can appreciate this is normal. Normal salmon--abnormal salmon, all abnormal. So this thing was first detected in Norway in 1999. It then spread throughout Norway, now it's in the UK. It's in Chile and it's killing a lot of fish. So what we did then was to take a high throughput sequencing approach and try to identify a novel arenavirus. But we were only able to find some of the segments of this virus. Now typically, viruses of fish, known as aquarena viruses have ten or eleven segments, and we were always able to find eight of those, and the ones that were most interesting to us as vaccine candidates were missing, so by using the approaches that I showed you, the cryptographic approaches, that my mother thinks are no big deal, we found these two which are now being used to make a vaccine. So you can use these approaches. They're really quite helpful.

Now this is a virus we discovered shortly before Thanksgiving last year that was infecting turkey poults. The question is then, how do you prove this is linked to disease. The reason I'm showing you this is to show you how rapidly we can do this, not because I care a lot about turkey poults. The point is that we solved this one in 48 hours. We took sera from the turkey poults, demonstrated that they if would label sections from the bird that had the disease, and then we used this method for a T2 hybridization that allowed us to simultaneously demonstrate the specificity of what we've found in terms of sequences and its capacity to cause damage. So the unaffected animal is shown here. The animal with disease is shown here. So the viral probe is quite specific. You don't see any signal here at all. And this probe which is actin, which is the normal housekeeping gene, is dramatically reduced to appear with the normal data, so with one 4-panel figure you can demonstrate the specificity of what you found for the disease, its effect on the tissue that's disturbed, and the whole thing from soup to nuts took six days. So it can move very, very quickly, but I'm not suggesting it's all going to happen that way for you. But it can happen.

Now sometimes we're asked to investigate things that look like infectious diseases but are not. This is work we did with the Minnesota Department of Health, that recently got a prize at the CDC. You will be able to see in a moment.

So, there were people who were working at a pork processing plant in Minnesota, and they began having difficulty staffing the plant. So I was asked to take a look at this vis-a-vis it being an infectious disease. When we went and looked at the plant we learned something very interesting. Every place you see a blue rectangle tis where there is somebody who worked on an assembly line who did not have disease. Every place you see a yellow rectangle is where you did have disease. This area here is where the brains are removed. This is where the brains are packed. If this were an infectious disease, you would have seen a cluster here, then a cluster here, then a cluster here, that sort of thing. This looks much more like a sort of a dose response, where, see, the closer you are to the offending fact, the more likely you are to have disease. Now when we began to figure out why this was happening, we learned the following. Somebody had decided that there was a real call for scrambled pork brains

in Korea and China and, because they were processing 30 thousand pigs a day, they were cutting off some bits, you know, the choicer bits here, and then they were collecting the brain material, but it's kind of difficult to scrape up the brain, so this is a high pressure hose similar to what you would use to inflate an automobile tire and there's the pressure plate, here is the eye of the pig, there's the nose of the pig, and what you do then is they take that hose against the pressure plate and pull it in, like so, the air flies into the skull, it scrambles the brain, it runs down this groove, and it gets collected. And here's one brain that's been processed, here's the next one ready to go, you know. And they were doing thousands of these things a day. 30 thousand a day.

Now you'll notice that these people have, you know, bare arms here. They had no protective equipment. So they were developing what looks like experimental nervous neuritis, an immune disease, due to exposure to large amounts of pork. And porkare are very, very similar to humans, so when you go to the South Sea Islands, for example, in the old days when they used to eat people, they had a long pit and a short pit. We're long pit, and that's short pit. Anyway, we won the Charles Shepard award last week for that. So that's cool.

So what we found is that there were antibodies directed against the peripheral nervous system. This is some work done by Mady Hornig, again looking at indirect mechanisms by which agents can cause infectious disease.

Several years ago Sue Swedo Bennett at the National Institutes of Health recognized that there were a group of children who had obsessive compulsive features. They would collect their saliva, they wouldn't eat, they wouldn't step on cracks in the sidewalk, there were all kinds of things that they would do that were just strange. And they were coming into a schizophrenia clinic. The chief office looked like a disease. It had originally been described in the 1600's by John Siddon where people had weird choreia-forming, choreia in Greek means dance, in association with this obsession, in association with cardiac murmurs, in association with streptococci. So she decided that she ws going to treat these people with plasmapheresis. And she did plasmapheresis and sham plasmapheresis. And the kids who got the real plasmapheresis improved. Believe it or not, she did a double-blinded trial of plasmapheresis. Hard to imagine that you can do that, but you can do things like it at the NIH, right Frank? It's amazing. Hard to believe that. But we looked at these samples, and we couldn't find anything that would explain this, so what we did is decide to build an animal model. We took mice that were highly susceptible to inflammatory diseases, we immunized them with killed strep and these animals then developed all of these repetitive behaviors as you see here. They tend not to explore their cages. Here's a normal animal walking around, here's an animal that doesn't do it. They tend not to socialize, they tend to avoid the other animals, and when we pulled antibodies directly out of these animals and put them into other animals, we could replicate the entire syndrome.

Then we went back into the brains of animals with disease, we pulled out proteins that were complexed with the antibodies, we microsequenced them, and we cloned and expressed them. We went back to the children, we found that fifty percent of the children reacted with the same protein, the human humalog, as the mice reacted to. So now, for the first time, we had a biomarker for this disorder.

Now, this is something I call de-discovery. This has to do with MMR. We became interested in the idea that MMR vaccine might be associated with autism because of the work that had been reported by Andrew Wakefield where he had suggested measles virus was going into the intestinal tract, increasing permeability to toxins, opening up ___ made by bacteria, and he then had a series of reports that came out. Despite a number of epidemiological studies, nobody actually went back and looked at tissues directly, so we did that. We did a blind analysis and we found no correlation. We didn't stop there. We decided to ask another question, and that was, why are these kids sick? Because at the time that we started this work, very few people appreciated that 25 percent of kids with autism have severe gastrointestinal problems. Those kids with severe gastrointestinal problems frequently look relatively normal until about 36 to 40 months of age, and then they undergo some sort of deterioration.

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Now, I'm going to give you our hypothesis and so forth as to how this works and where this might go. But I have to tell you that some of this is speculative.

This first cartoon illustrates how the gastroentestinal tract works. So food comes in this end here, there are a number of enzymes that break down sugars, and then there are molecules that break down sugars, transport them across these enterocytes to the cells that line the bowel, then back out the other side to the blood.

So using a method called transcript profiling we looked at genes that encode the things that make these enzymes that break down the sugars, that carry the sugars, and the control molecule that controls them all. What we found was that the children who were autistic--this is the AST column--there were dramatic reductions in the enzymes, as you can see, that are responsible for breaking down the sugars. And the transporters were also dramatically reduced. But this is a housekeeping gene that was not. So then we reasoned, okay, well if they're not breaking down the sugars, and they're not transporting the sugars, there probably will be some sort of effect on the distal microbile. Right? Because there's all this sugar there. So we began to look at different populations of bacteria, PROM acute, proteobacteria and so forth. And remarkably, when we began to dissect this further, we found that in kids with autism there was a particular bacterium called sutterella, which is not present in the others. So now we have candidates. We can go back and try to examine hypotheses like this.

So this may give us an explanation for why some people respond to dietary restrictions, some people respond to probiotics, why and whether or not this has links to neurological symptoms remains to be determined. But with data like this, you can begin to take apart the model and figure out what will and will not fix this.

Now, I began this kind of work really in the early 1980's initially because I was impressed with the fact that it took so long to figure out what was causing AIDS. And then subsequent to that, I began working on another virus which was called bornavirus, which was also eluding characterization. But the more I did thinking about Central Nervous System diseases, the more it became clear to me that in their final common pathways by which a wide range of organisms might cause disease--animal models proved this, epidemiology proved this as well. And then we began to look at data that suggested that common components of bacterial cell walls had double stranded RNA as a model for replicating viruses might cause disease. These, again, are mouse studies, but mice are very useful for at least beginning to at least generate hypothetical hypotheses. The typical gestation period for a mouse is 18 to 21 days. So roughly halfway through the gestation period of a mouse, if you expose it to either influenza virus or to polyinosine cytosine, replicate ... mimicking or replicating RNA virus, this animal won't run around its cage like a control animal. So this is a track bot over 15 minutes and it covers a lot of ground. This animal, in contrast, just sits there. If you do the same thing a few days later, same thing, same genetic background, same stimulus, the animal becomes hyperactive, so compare this animal with this one, and this with this. So, the message is the same genetic background, same factor, different timing can give you a vastly different effect. And this can give you some explanations of logic for thinking about timing with respect to pathogenesis.

So how does this work? So this work recently published by a colleague of mine now in Brazil illustrates the effects of viruses and presumably bacteria as well on replicating neural stem cells. So the Central Nervous System develops from the inside out. We have these cells, which are stem cells, which are sitting out here before, and then over a period of time, they give rise to neurons directly or to these green cells called intermediate cells and then they eventually get this nice architecture and so forth, and then the synapses form and then you get a functional circuit. You get a real hard drive. So what Jawahar decided to see what would be the effects of introducing poly(IZ) or polysaccharide or infloexebar on these populations themselves, these neural stem cells. And what he found was... this is a facts plot ... it doesn't make any difference how it works, but all you really do need to appreciate here is that ten to the fifth cells, which is illustrated in red, which is where you have a phosphate a buffered saline, which is a normal control, and there is a one order of magnitude difference, ten times fewer cells in the animals which have been exposed to the virus or the polyinosine cylosine. So as a result of exposure to a virus, you're losing ninety percent of these cells. It's a very, very dramatic effect, and the specificity of the effect became clear when we used a

specific type of mouse, a genetically modified mouse that cannot respond to poly(IC). And here there is no difference between the animal that receives the poly (IC) and the animal that is the normal control. So it's a very specific effect.

And now this gets back to a question that you were asking about anti-inflammatories. So if I know which animals are at risk for disease, and I give them an anti-inflammatory drug, im this case it's an non-steroidal anti-inflammatory drug, there is again no difference between the animal that received the poly (IC) or the virus and the control. So it's the same thing. So, again, compare this, with this, with this. So with that, we can identify who's at risk, we can actually have a hope at really preventing this disease.

So the next few slides, I'm going to talk about, you know, what the future is for this kind of work, and this is a nice one.

So, web based surveillance platforms. It may not be this important in thinking about chronic disease, but perhaps it will. You can leverage social media to obtain insights into different kinds of diseases and see how people are responding to different medicines, to drugs, and that is ...

Sequencing is dramatically changing the whole landscape. It's becoming cheaper and cheaper all the time. I've heard people talking today about wanting to do that with genetics, but, you know, it's still out of range, unless you want to do, you know, a hundred thousand dollars for every single workup for every patient. But in five years, that may not be the case. We'll see. So, we have to be patient and wait for some of these technologies to move forward. As I showed you, the biggest single factor in the growth of the viral sequence database was, in fact, improvements in technology. It has been moving forward in a logarithmic fashion.

There haven't been many people who do much with serology because it's more difficult. People can make slides with oligonucleotides and do green chips and vira chips and everything, but it's more difficult to get at the immune response.

Sample preparation is something which is key and I'll talk to you about that in a moment.

And then thinking about gene environment-time interaction. As I've illustrated for you, when you see something is very important.

This is not too important. But I will tell you for synthetic biology and the ability to make biological weapons is very much on my mind and on everybody's mind right now because it's cheaper and you can buy all the equipment on Ebay at this point.

Now when I originally undertook this work, and this is the first agent that was isolated using purely molecular method, this represented my entire fellowship. It was six years of hell under Michael Olstrom, for which I'm grateful because it allowed me to get out clinical medicine into this. I shouldn't say that. Probably a lot of you are clinicians, but I'm very grateful to be in the search because it's been a lot of fun.

So for the last six to twelve months, we have literally discovered hundreds of new viruses, so where that took three to four years, this now takes a matter of weeks. Now until recently, I don't think arrays were ready for implemention on pathogens but I discovered that they are really improving dramatically. This was early work. What we did with this back in 2005, we were looking at viral hemorrhagic fever. In this instance, it was a Marburg virus outbreak, and there was somebody who was part of the team who became sick and died. And the question was, did he have Marburg virus, which is like ebola, and he was tested in the field and he was tested in Atlanta, and nothing was found. We took him on the green chip, and it was found that he was infected with malaria. It's a horrible story. I mean, can you imagine dying of malaria? You know, he didn't have any malaria prophylaxis, but that varies.

Now things have changed dramatically, so now we have the ability to move past the sensitivity of ten thousand copies, it's automated, it's quick, we're looking at defining the various regions of the genome, it's going to revolutionize what we do.

Hotspots of emerging infectious diseases. You will hear about this. You will hear about hot spots of Chronic Fatigue Syndrome, hotspots for Gulf War Syndrome, hotspots for all sorts of things. This is an effort to leverage some of this media, social media is what we're trying to understand, so you'll note that every place on this heat map which is red or orange is an area where there's a lot of diseases. So these are the areas where we consume a lot of bush meat. Here's an area where we have a lot of other problems with over-population. And if you look at other parts of the world, you know, you begin to see you have large migration effects and so forth. So there are a number of factors that are important. Agricultural intensification, climate change, bush meat, human susceptibility to infection and so forth. So we're modeling all of these possiblities and we're studying them.

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So what are we finding? Well, this is an example of a novel filovirus. Filoviruses include Ebola and Marburg virus. The first one that's ever been found in Europe and these were in fruit bats located in Estonia and northern Spain. Now why is this important? First of all, the idea that there's an Ebola-like virus circulating in Europe that we didn't know about is a frightening concept. Number two, the distribution of this particular bat is quite broad. It includes most of the southern tip of Africa, it goes throughout Asia, it goes through southern Europe and so forth. So if this particular virus gets loose, it can affect all of these areas. And this is simply an area where it's located and so forth.

So here is this virus, called Loco virus, after the game from which it was identified. See the Marburg viruses, the Ebola viruses, paramyxovirus and so forth.

Is Sergei here? Okay, so that's a kind of a cool story, isn't it? Yeah, see.

So, here's another one. This was a virus we found in Bangladesh which, at that point, was the closest relative to Hepatitis C. It was identified by Jon Epstein with the help of ___ but more recently we found something even more striking and closer to home.

Now, though dogs are man's best friends, they separated from us in every way in ancient history, some 60 million years ago. They joined us again about ten thousand years ago. At that point we were eating them. Right? So you see a dog skull fragment in human feces. So, in any event, nonetheless, they've been with us since that time. We're investigating outbreaks of respiratory disease in three states, and what we found through high throughput sequencing was this novel virus. This is the GBV virus that we found in bats in Malaysia. This is the closest living relative now to Hepatitis C. Now, when this was published, people said, you know, we have to kill all the dogs in shelters because, you know, we're going to have more Hepatitis C, but in fact that event happened a very, very long time ago. This event is probably specific.

Now I mentioned something very early on about that fact that when you're sequencing out of tissues, or cells, the majority of what you're going to see is going to be host. It's not going to be virus. It's not going to be ___ materials. So the question is, how can we around that? What we've done is we've gone through every single high throughput sequencing exercise we've ever done. We've identified the primers that gave rise to sequences that were human. We've removed them from the pool, we've then gone back, this is from the Lujo virus that I showed you, the one that killed all these people in South Africa and in Zambia, and you can see that there's very, very low coverage here, of this particular genome.

This was the new, improved method, so this is less than 10X, and here you can see it's more than 220 X. What does this mean? It means for the first time, if something is present in a very, very low copy number you can see it. It also means that it's cheaper to do the work because you can do 1000 times more work for the same price.

So you can begin to look at cancer, autoimmune disease, diabetes, encephalitis and so on. It's just a question of your imagination, how far you want to go.

We are trying to develop a serological platform that will allow us to ask the same kinds of questions that we do with genetic assays. It is less well developed, but it will move along.

And this is some work we're doing in Norway with a large unselected cohort to address that whole issue of cause must precede the effect, so ... and there may be sometimes distal effects. You may be infected with something now, and 20 years from now, you have MS or chronic fatigue or cancer, what have you. So what you're doing is you're tracking people through life as they move on. You go back and look at prenatal materials to see what you can duplicate and that's important.

And we're doing work with multiple laboratories in support of the international health regulations ___ and so forth. And lastly, I'll close with this. We're making a movie that'll be out in September or October with a very, very good cast and it features a new virus. It's not XMRV. And I'll close with that. Thank you very much.

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Q & A

LIPKIN: I'm told that there's a very specific way in which I'm supposed to handle questions. There are microphones...there they are. Okay.

So, are there any questions?

Well that was either clear... or ...

Q: I have a question. Yes. With regard to the compound being some days more prevalent in someone and other days, there's not quite as much of that, and also how does that affect cognitive function?

A: I'm not a clinician taking care of patients with Chronic Fatigue Syndrome. So, I'll have to turn that over to somebody who knows something about those aspects. Is there anybody else who'd like to know?

(unintelligible)

You're going to answer? Well, take your time. Please. ... Take the mic.

(unintelligible)

Q: ___ to get through that sometimes ____something that takes longer, not how ___ days, weeks ____

_____

A: I could speculate if... if I want to say that there's a virus responsible, like with herpes virus infections, for instance, it's not unusual for there to be flares, When there's a flare, there's a cytokine and chemokine response. These are chemicals that come out into the blood. They have an activity in the Central Nervous System. They may give you confusion. They are associate with fever, and so forth. This...

Q: (unintelligible)

A: Well, either take the mic or don't but to me that's so, I think the best example I know of that sort of periodicity, a couple of other things, there are some animal diseases like brucellosis, which is a possibility too, but there are a couple of things...and malaria also. There are some that ... they have a

periodicity, just as you described The agent is latent. It then leaves or it's sequestered, it then becomes more generalized, and then it gets contained again, presumably by the immune system or by the innate immune response until it blossoms again. A classic example of that, the best one, really, would be herpes infection, particularly herpes simplex type 2, where people do feel systemically ill, with pre___ do anyway, particularly early in the course of the disease. So something like this might be operative in chronic fatigue. I don't know about it. I don't know the agent. But if it's a herpes virus or if it's a certain type of bacterium and so forth, that could explain it based on ____.

Q: Could you talk a little bit about the pitfalls of PCR diagnostics? I know you've been using it. You were one of the first to use PCR. It obviously has great utility, but can you talk...touch a little bit on the pitfalls of using PCR as the be-all, end-all of diagnosis?

A: Well, the problem with any very, very sensitive method, probably, is that if you have any small amount of contamination, you will see it, because you are pushing the gain up very high. And there are measures that you need to take to mitigate--you never entirely eliminate it, but to mitigate it. So the mechanism of things that people will do is that, you know, they don't work with an agent in the same place they're going to be surveying for it in the normal population. They put ultraviolet light in the ceilings. They use positive pressure environments to assemble the components to the PCR. They create working stocks that they use only once. So, there are a number of...I mean, we've done a lot of this because we've done it with Borna and with other viruses in ALS and MMR in Autism and we're not doing any work at all on Chronic Fatigue Syndrome at present. But this is not an area where anybody can go, frankly. I think it requires specific expertise, attention to detail, and strict hygiene, far beyond what we need, for example, in an operating room.

The best example that I can give you is that the type of hygiene that we use to do this kind of work is like what you use in Silicon Valley for assembling computer chips. Because, you know, a little bit of dust and your cell phone doesn't work, or your computer doesn't work, so these are hygienic environments with scrubbed air and so on.

(To Annette Whittemore) You can ask a question any time.

Q: Robert Miller. Uh. Um. Over having a ____ for over 25 years, what are your thoughts on patients who basically upset the patients becoming sponges for viruses like CMV, Epstein-Barr or HHV-6?

A: I'm not sure what you're really trying to ask.

Q: Just... at any particular time that ... um... you're tested for one of those particular viruses, um, there may be times that you have a high titre, but there may be other times when you'll actually have active infection going on, and it doesn't seem to be... it's not... one of those particular viruses, it just runs the whole gamut.

A: So, I talked a little bit about this, this afternoon about the late 1990's we did some work with the CDC. It was specifically on Borna virus and its potential role in Chronic Fatigue Syndrome. And we found no evidence to support that, but we did find by my colleague, chronic T-cell activation. That is to say that patients with Chronic Fatigue Syndrome, something like 77 percent in the survey that we had access to had antibodies that were reactive with a wide range of different proteins. And there were two interpretations to these data.

One interpretation was that the bulk of these patients don't have the infection. I'm not saying that was mine.

And my reaction was that was wrong. I draw the opposite conclusion. I draw the conclusion that these people have some sort of immunological problem which may be infectious in nature, maybe not, maybe genetic. I don't know. Maybe toxocological. I can't say. All I know is that they have immune systems that are hyperactive. They're clearly abnormal, and it also allowed us to say, here's a biomarker that may be useful in saying we can track response to medication and for the elevation of the immune system for purposes of social security or disability or whatever the case may be. So I

think that there's evidence that suggests that there are a wide range of different presentations with Chronic Fatigue Syndrome. Ssome people look like they have a smoldering disease. Some people look like they have a disease with a lot of consistency to it. Some people have a very aggressive downhill course. I don't know that these are all the same in origin. They may be different.

So one of the things that we need to do, I think, is to try to find a way to parse these differences, and then to consider the possibility that they may be different.

I don't recommend, however, that you sort of parse yourself out with one advocacy clique for this aspect and that aspect. You've got to stand together to get any sort of attention, but I think that it's going to be a more complex answer than simply one agent.

We have another question back there. All the way back.

Q: ___ your hypothesis that there are undescribed viruses that

Man in audience: You have to use the microphone.

Woman in audience: There's a button on the microphone.

Another woman: If you push that button, the microphones go on automatically.

Q: Can you hear me now?

A: Yeah.

Q: Got it. Is it your hypothesis that there are unidentified viruses out there awaiting discovery through high throughput sequencing identifying them. I thought disease distribution is heavily biased toward discovering infectious disease viruses. What about transforming viruses that can cause other... having to do with cancer? Do you think this approach would work for that? And do you think there are unidentified transforming viruses out there as well?

A: Possibly. I'm a little uncomfortable with the way you posed the question, so you said my approach is biased. What would... You know, in science as in everything else, we have to apply for money to do certain kinds of things. So there's a lot of interest in the notion that there may be acute threats waiting and threatening humankind. So there's a lot of support for doing that kind of work. And, with the money that's left over, and it's not much, frankly, you can then go and approach chronic diseases, but there isn't a lot of enthusiasm, except in verbal discussions, you know, for supporting a big search for infectious agents in chronic conditions, whether it's the neurological institute, child health or mental health or National Cancer Institute. So there's literally, the USAID Predict program, for example, which is something like 20 million dollars a year, just for sample collection, something like a total of 75 million. And the Department of Defense--all this together is probably a hundred million dollars a year that's going into those kinds of projects. We're trying to piggyback on those to do these other kinds of things, but the amount of money that's available, through NCI, for example, is paltry. It's 250 thousand dollars. You can't even begin to do a rigorous search for that kind of money, you know. It costs you 50 thousand dollars to do, you know, a really good run of high throughput sequencing, then you have to crack all of those leads and find out which one was real. Then you have to make antibodies and do all this other stuff. I mean, every discovery that we have, that we really take through to completion, is several hundred thousand dollars. So, until there's a change in the mindset of what gets funded and how it's done, I don't see a lot of hope for big investments in that arena. That doesn't mean we're not interested in it, or that we're biased in our approach. It simply means we don't have the resources to do it. And every time I talk to a group like this one, I'm hopeful that somebody is going to walk up to me after the end and say, "This is really terrific. I would like you to do this...in type 1 Diabetes Mellitis, Chronic Fatigue Syndrome, Parkinson's Disease, and I will write a check for a million dollars." Anybody who wants to write a check, I'm not being cavalier about this at all, I'm really serious because if somebody can pay for it... but you know, we have to pay for the sequencing, we have to pay the salaries, we have to pay the bio___ for the

work to do it. So if anybody wants to take this in any disease and not just me. I mean, there are several people who would undertake this for you. But there are no resources to do it. That's the challenge. So, again, I'm not being tongue in cheek, I'm not being flippant. I mean it sincerely. If we have the resources to do it, we can certainly do it.

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LIPKIN: Sergei.

Q: There's some comment. I mean, there is another part to it, actually. The same reasons, the same situation, a sizable portion of those sources, right, come from rather unusual sources, not from the usual population, for ___grades sometimes retrogrades, can you ___ of some ___ of those viruses in the wild, and if you unlock its secrets as you do now, and your viral databases is having it, and you find something similar that's similar, that's ____, you have its secrets so you can catch anything, I mean, like something unusual and it's not in the database.

A: Well, Sergei that's why I showed you ...

Q: (Sergei: unintelligible)

A: No, no, but we ... those databases have been made, they are available. So the one we used for the salmon is called FASDEN(?). You can get to that directly from our website, and you can take your sequences and download them and run them, and it will give you ... What it will tell you is that there's a probability that what you found is more likely to be a double-stranded RNA virus or a positive strand RNA virus... These tools are there, you know, for use, so you can access those. Now,

Q (Sergei: unintelligible):

A: No, no, no, no, no. Not philogenies. What it will do is it will give you a statistical significance for it, an say it's more like this than like that. Now then what we typically do is the following: you look for the usual suspects, right? You don't find the usual suspects. Then you take your window in a blastex, look as best you can. If that doesn't work, then you relax the conditions and look again. And if that still doesn't work, then you go to FAS-D, and you ask yourself the following question: Does this look like positive strand RNA or negative strand RNA, whatever. And then what you do is you take all the sequences that you have that fit those parameters in FAS-D and you ask yourself, if I were to create a scaffold, what might this look like. Right? Would it? Could it? And then you do it every which way. You know how to do this. You look at it with this primer here, and then you do it with this one. And then you do it this way, and you do it this way, and you do it that way. And eventually...and you get a product and you sequence it and you move on from there. It's just tedious and it's hard work. It's like sequencing the first ____ virus. It's that kind of thing.

Please.

Q: What Dr. did you say about ___ all the more to do good research and a crime ____ . Much as I hate to say this, and everybody in this room owes a debt of gratitude to the Whittemores because they spent the money to move this kind of technology ahead.

(Everyone claps, incuding Lipkin)

Q: Will you tell us what the (unintelligible)

A: Colonial 123 drug. So the project that we're doing now with the Department of Health and Human Services is not really a Columbia project. It is a project that is centered at six clinical sites, three laboratory sites, one of those sites is WPI. We're the coordinating center. Absent support from a philanthropist in New York, who is interested in looking at biomarkers from well-characterized patients, and for doing pathogen searches using the kind of technologies that I've described, and we have support to do green chips and we've developed Mass-Tag PCR panels that will be used to screen

these patients, and there is a subset that will have deep sequencing done. So assuming that that agreement proceeds, we will focus on biomarkers, associated with, you know, acute disease, relapses, remissions, as well as looking for pathogens.

Q: There will be ___ (unintelligible)

A: I don't know anything about Dr. ____. I'm not involved with that.

Q: Can you just comment on your study?

A: So the study that we're doing, of which I said, WPI is a participant, the results are going to be unveiled to the participants, and you'll have a representative there who will see those results at that point, that group is going to have to decide, I mean I'm not going to let them not do those results. Right. On the other hand, it's not going to be like the Oscar's night where it says "May I have the envelope please."

Please.

Q: Dr. Lipkin, I'm a patient with CFS. If I had the money and I give it to you right now, what study would you be doing on what the cause of CFS is?

A: We would do deep sequencing and proteomics on sera from people with well characterized disease at various time points. Before and after stress tests, whatever we could do to try to increase the level of expression of the agents.

Q: Now that you've said this, do you feel that this is something that you suspect the agent of being viral?

A: The thing is, to the guy who's holding a hammer, everything looks like a nail. So, you know, I'm a virologist. So it looks to me like a virus. But I also like, I mean, I also work with bacteria and fungi too. But it smells more like a viral infection. But it would not at all surprise me if it were a common viral infection to which people had an uncommon response. There are all kinds of models, but what we prefer to do is to see whether or not there's a consistent finding, you know, in some subset of people.

This will be the last question.

Q: Will you, or have you ____ in mapping out the current ____ or have you not raised this____

A: What are we referring to?

Q: ___ past heart attack, no _____ but you were also saying the year 2009. There's a program called Gap Finder where all you have to do is look for the ____ position. It doesn't matter. It takes like...it's great...it's like ... for example, ___enterococcus from _____ the defect ___ okay.

A: There are a number of .... The question is what sorts of algorithms are now being used to track the changes ____ in its various diseases.

There are a number of different programs. There's one out of Boston Children's Hospital called Health Map. There's a Gideon out of Health Canada. There's another new one sponsored by Google. I can't think of its name right now--Google Trends. There are several. The problem with all of them to date is that they generate a lot of signal, and somebody has to go and do the gumshoe immunology and follow them all up because a lot of the leads turn out not to be fruitful. If you go backward, however, in hindsight, you can see signals for SARS prior to the first recognition of it by the WHO. So, the systems are not as sophisticated yet as they ultimately will be. If you go online today, you will find the first report of the National Biosurveillance Advisory Subcommittee, which I chair along with Jeff Engel, from North Carolina. This is the first step on the part of the federal government to come up

with a concerted program for investigating incidents of diseases, infectious disease, toxins, threats to food supply and so forth. And in that we specifically call out for investments in bioinformatics, use of common terminology, as well as social media to try to get at these questions. So I think you're on the right track. I don't think the systems are available to do that. I think, in fact, they stink. So, we've actually said specifically that the federally government should have a GARPA-like program to address specifically--and I agree with you--what you think we need. Because I think that is, indeed, what we need. It does not exist. And to get people to work on that, again, you're going to have to have, you know, an X price, to come up with some sort of a system that's going to allow us to recognize these events and have the signal rise above the noises of the ____. So when I talked about the future and I talked social media, I think that's extremely, but I work with WHO and I'll tell you this, we're not... we're still operating the old way. We're operating blind. We require reports from ___ countries to tell us that there's something going on. Something named Pro-Med, and there's another one called the Global Operating Alerts Box Network, which also releases these kind of data. But if you have talents and you approach ... Are you a programmer?

Q: (unintelligible)

A: Okay, so if you know of programmers who want to get involved in this field, we're desperate for them because we don't have them, and we've tried to appeal to people like Ervel, you know, who used to be at microsoft, and there were some people at Google who were going to help. They're not doing very much any more, so unfortunately, there aren't many people doing that kind of work. But we need it. We really do need it.

Thank you very, very much.