

Panel VI: Case Studies – Prevention and Treatment

Moderator: Huntington F. Willard, U.S. GDEST Program Committee

. . . Institute for Genome Sciences and Policy at Duke University in the United States, and had the pleasure of being on the program committee for this conference and will chair this afternoon's session.

Our first speaker is Professor Wei Lai, from the Hepatology Institute at Peking University who will be speaking to us on hepatitis B.

Wei Lai, Health Science Center, Peking University

HbeAg Negative Hepatitis B, Mutation Genotype, Genotyping Relationship, and Outcome

Chairman and ladies and gentlemen, good afternoon. In China, the hepatitis B and all hepatitis such as hepatitis A, hepatitis C and belongs to the infectious disease. If they have a different transmission route . . . hepatitis B and C, most patients . . . transfusion. Sometimes it was an unsafe injection.

Here I want to talk about a new concept, HbeAg negative hepatitis. Most of the patients fall under hepatitis B, or the positive HbeAg. However, patients to have the higher . . . and higher DNA, . . . but they have the elective HbeAg. So, what is the relationship for the HbeAg negative to the genotyping and That is what I want to talk to you about this afternoon.

First, when you talk about genotyping, I would like to introduce the geographic distribution of hepatitis B virus genotyping in China. China is a huge country and the different areas have the different prevalence of hepatitis B. Also, for the difference to hepatitis B genotyping . . .

In this slide, we also concluded some of the large cities in China In some areas, most patients . . .

(not transcribed)

Moderator – Thank you, Professor Wei. All of the speakers will take questions at the end as we have done in the other sessions. Our next speaker is Dr. Steve O’Brien from the National Cancer Institute who will speak to us on genetic architecture of complex infectious diseases – lessons from AIDS.

Steve O’Brien, National Cancer Institute

Genetic Architecture of Complex Infectious Diseases: Lessons from AIDS

Thanks Hunt. It is great to be here and while I’m hooking up, I’d like to thank all the organizers listed behind me, but if I did, it would take up all my time because there are a lot of organizers, for allowing me to come here.

Halfway across the world and maybe a generation ago, the species conservation community learned an important lesson about the role of host genetics on the outcome of infectious diseases from an unlikely endangered species – the African cheetah. The species as, at the time, unusual because it had been shown to have survived a near extinction event some 10,000 years ago, which had homogenized its overall genetic variation intensely. But, notably, the genetic

variation that is involved in immune defenses, the different armaments which equip species to defend themselves against infectious agents they have seen in the past as well as new ones which are invented during their lifetime. An outbreak took place in the most successful breeding facility of cheetahs in North America, a small drive-through park called Wildlife Safari which had close to 100 cheetahs breeding successfully. A couple of animals were brought in and became ill while in quarantine, and died of a disease caused by a corona virus, a relative of the SARS virus called feline infectious peritonitis. The virus spread through the park within six months and infected nearly every animal in the park, leading to the increase in antibodies against the virus, as is shown in this family. Before the arrival of the first two cheetahs that were infected, all the animals were negative. But, within a year, everybody had become infected. There was not a normal stool in the park. All the animals had diarrhea. All the animals had nervous twitches. All the animals had jaundice like hepatitis. Within six months, 50% of the animals were dead and 70% of the animals were dead within three years. It was the worst outbreak of feline infectious peritonitis ever seen in any species.

Twenty years later, in the Gwong Dong province of China, a flu-like syndrome developed into the SARS epidemic late in the year 2002. It spread to 29 countries, and inflicted enormous emotional, cultural, and economic pressure upon many countries, particularly China and other Asian countries. When the epidemic burned out at the end of July, 2003, there had been a total of 8,000 people infected, and 700 deaths, a mortality rate of about 10%.

The SARS virus was a member of a corona virus family which we knew about because corona virus had been described in domestic animals, and had also been responsible for about one-third of human common colds. When the sequence was analyzed in a phylogenetic method, the SARS virus however did not fall in any of the traditional clusters of avian corona virus, or human corona virus related to bovine and mouse hepatitis virus, but rather came as an off-shoot which was distinct because it was evolving probably in a different host or reservoir species recently identified as several species of bats.

The cheetah virus, when sequenced retrospectively, turned out not to be a relative of the SARS virus, but to be almost indistinguishable from the domestic cat virus. The difference between the

mortality and the morbidity in cheetahs and humans was probably something to do with the genetic uniformity of the cheetah species because the mortality in humans, as bad as it was, was only about 5-10% and in outbred domestic cats, the same virus has less than 2% mortality. But, in cheetahs, this high uniformity is probably a consequence of a virus that overcame the defenses of a first individual, looked around and found the rest were immunological clones of each other, leading to a rather uniform homogeneous response. The genetics of the host can make a difference in how well a species does when it incurs an infectious agent that is deadly.

Like many historic epidemics, HIV/AIDS is very deadly. It does not respect geographic, ethnic, cultural, or social strata, but even so, displays some considerable heterogeneity of outcomes among different people. Today, it is my pleasure to present a brief overview of the genetic variance, AIDS restriction genes, or ARGs, that regulate an individual's response to HIV and infection. I will attempt to highlight a bit of the gene discovery process, the mechanisms by which the genes act, the implication of host genotypes on the progression of the epidemic, how the genes may help inspire new therapies, and the process for hopeful but not quite yet ready for prime-time future discoveries.

To begin with, I shall look back a decade, to 1996, the year that two similar discoveries gave us some needed hope for the horrors that we had known as AIDS. AIDS, of course, was first discovered in the early 1980's, about the time of the cheetah outbreaks that I described a moment ago, and led to ten years of a steady increase in the number of mortalities, of an incurable disease which still today we have no vaccine and no effective treatment.

The discoveries that came in 1996 were two. The first was the application of the powerful, evolution-inspired strategy of triple drug anti-retroviral therapies. HAART – highly active anti-retroviral therapy which for the first time actually caused a decrease in AIDS mortality, at least in the west, and the mortality had been steadily rising up until that time, since it was first recognized 15 years earlier.

The second discovery was a little bit more arcane. It was the contemporaneous announcements within a week of each other in July 1996, in back-to-back articles in *Science*, *Nature* and *Self*,

written by five outstanding research groups which identified CCR5 and CXCR4, heretofore innocuous . . . receptor molecules, as the required portals or doorways by which HIV enters macrophages, monocytes, and T-lymphocytes. The ligands which normally bind these chemokine receptors, CXCR4 or SDF1 physically interfere with the binding of HIV and HIV entry. That was how the receptors were identified.

By this time, our group had assembled a consortium of exquisitely curated AIDS cohort populations, extracted their DNA, and were screening human variance that might explain why not all exposed have become infected and why not all infected had developed AIDS. These included patients in epidemiological cohorts in the risk of group of men who have sex with men, homosexual men, hemophiliacs, who receive contaminated clotting factor before the implementation of the HIV blood test in 1984, and IV drug users who shared needles in city slums where the HIV incidence in that risk group population approached 80%.

Well, we, and others, rapidly re-sequenced the coding genes for the chemokine receptors and the ligands to discover CCR5 delta 32, a natural genetic knock-out deletion that was carried in one in five European Americans, but was never seen in native Asians or in native-born Africans. People who carried two copies of CCR5 delta 32, between 1% and 2% of all European Americans, were near completely missing from HIV-infected people, suggesting that the CCR5 molecule on the surface . . . lymphoid cells, was a necessary requirement for HIV infection.

As illustrated in this slide, the frequency of homo. . . for delta 32 varies between 1% and 2% among European Americans, but is nearly completely absent in HIV-positive people because people without CCR5, people with two copies of delta 32, one from each parent, simply do not have an entry portal by which HIV to enter cells, and are completely or near completely resistant to HIV infection no matter how many times they become exposed.

People who carry one normal copy of CCR5 and one deletion copy do become infected. However, they progress to AIDS-defining conditions slightly more slowly, as is illustrated by the survival curve where sero-converted patients are plotted at the rate at which they go on to

develop AIDS, and individuals that are heterozygous actually delay the onset of AIDS by two to four years, a modest and very slight difference, but it is statistically significant.

In addition, when we inspect the type of AIDS-defining conditions that people develop when they carry CCR5 delta 32, what we discover is that HIV-infected people show a difference in how frequently they develop non-Hodgkin's lymphoma. A B-cell lymphoma where individuals who carry delta 32 are twice as frequent among those who avoid lymphoma as those who are not. So, those three effects: a recessive effect on infection, a dominant effect on rate of progression, and a dominant effect on AIDS-defining conditions were the hallmark of AIDS restriction genes, as illustrated by CCR5.

Now CCR5 was interesting because, as I mentioned, it probably was born after the divergence of ancestor of modern Europeans from the individuals who migrated out of Africa 150,000 years ago, and then split to the Asian and Caucasian derivatives. In addition, the frequency of delta 32 shows a gene frequency gradient across Europe where it is highest up north in Scandinavia and in Scotland, 10% in France and Germany, 5% in Greece, Europe and Turkey, and 0% in the Middle East and Sub-Saharan Africa. This gene frequency gradient is suggestive of some sort of favoring or strong selective pressure in the north. In addition, the rise of a mutation along this lineage from a number of a very high number of people to up a frequency of one in five Europeans carry this as heterozygotes, was rather unusual because 99% of all mutations go extinct. So, several pieces of evidence led me to speculate, and my colleagues, several years ago that CCR5 delta 32 had . . .

. . . to her offspring to a frequency of one in five carriers in Europe. The evidences have been summarized adequately in the literature, but they include the fact that CCR5 is a knock-out mutation which eliminates a perfectly good immune function, but yet instead of being eliminated, it rose up.

In addition to that, a founder effect, which would explain a rise quickly, probably was not occurring in the timeframe we're talking about, but rather than European populations have seldom been less than 20,000 over the last 50,000 years. There is no evidence for a population

bottleneck like we saw in the African cheetahs. Delta 32 frequency, defined north/south gradient, a signature of selection, and finally, if you look at the pattern of variation in other sites in the CCR5 molecule, most of the mutations are amino acid altering. A signature of a favoring of molecules, as has been seen in the pattern of variation in HLA, the human major . . . compatibility complex, and not seen in other genes that are not selected by outbreaks of infectious diseases in the past. So, CCR5 seems to have been jacked up probably by infectious disease, but what was it?

The next key to the puzzle takes into account tricks that the molecular revolution have taught us, the ability to recognize that there is a timekeeper, a chronometer if you will, in the genes of all human beings, that can allow us to estimate how long it has been since a particular mutation took place, or more precisely, how far back it has been since a particular mutation that was selected was selected. How far back it was. The measure is the randomization of the single nucleotide polymorphisms that are adjacent to the mutation that occurred. The phenomenon of non-random association of adjacent variances, called linkage of this equilibrium. It has been measured around CCR5 several times. Originally we used micro-satellites back in 1999, as have other workers in most recently single nucleotide variance or SNV-based analysis have indicated that the size of the haplotype or the non-random structure around CCR5 is about a centi-morgan long. That centi-morgan long haplotype translates using a simple equation to a time or estimate of the date at around 700 years ago. This is the period that has elapsed since the last delta 32 or that chunk would have been selected.

Of course, this immediately led us to wonder whether in Europe, 700 years ago, that the black death may have been responsible for causing the favoring and the transmission of the delta 32 molecule. It was an interesting hypothesis. It was indirect, and it was guesswork. But, it was the best candidate we had based upon the timing of the mutation and the fact that everybody seemed to think the delta 32 had been selected.

The black death was a massive epidemic which took about one-third of Europeans in a four-year period between 1348 and 1352 in the 14th century, that started in Mesina, Sicily and it migrated throughout Europe in a bit of a circle, a little bit like the SARS epidemic except them it quite

abruptly, just like SARS. During that period, it took many cities up to 50% mortality, 60% in other cities. It basically then stopped. Several generations after the black death, there have been other waves of Bubonic plague caused by . . . that have passed through.

So, in addition to that, there have been waves of plague from several hundred years before, back to the Justinian period of the Roman Empire, which could also have contributed to selective advantages of carriers. If delta 32 actually had an effect on the cause of black death, which was . . . bacterium.

Having said that, there is not a lot of agreement that *Yersinia pestis* was, in fact, the cause of delta 32 rising up, even though we guessed that it might be. There have been other theories about what might have caused it, such as small pox, anthrax, cholera, Ebola, typhoid, even the Vikings having a strong influence. But recently, an interesting connection which connects *Yersinia pestis* directly to CCR5 was conducted by Stephen Albin and his colleagues and published in Nature last year. What they simply did was they took mice that were knock-outs for CCR5 and they isolated macrophages, and then they measured uptake of *Yersinia pestis* from cells that had a normal CCR5 and cells that didn't. What they saw was a 60-fold difference and a requirement for CCR5 for *Yersinia pestis* to be taken up, connecting directly *Yersinia pestis* biology to CCR5. I think this basically puts *Yersinia pestis* in the running in addition to some of these other candidates.

I'd like to change gears now and talk about some of the other AIDS restriction genes that we have been able to discover. But, before I do that, let me say that one of the reasons we were interested in identifying AIDS restriction genes was to point the new way to natural genetic resistance that might be useful for developing new kinds of therapy against this deadly disease.

Well, delta 32 discovery, which demonstrated that HIV's entry is necessary and can be inhibited by a natural mutation, actually has led to the development of a whole new generation of drugs which are different from the 16 heart drugs that are used right now. They are called entry inhibitors and this slide here is a summary from a recent discussion. There is a dozen of these made by different companies, all of which block HIV from interacting with CCR5 or CD4, or

facilitating the fusion process. Many of them have entered clinical trials. One of them, fusion or . . . , was actually approved by the FDA – the first non-viral drug ever approved for HIV. It is pretty exciting and I think the development of these things was precipitated and facilitated by the knowledge that delta 32 homozygotes don't become infected.

There are other kinds of human AIDS restriction genes that have also been discovered using the same approach since delta 32. We have discovered AIDS restriction genes that involve HIV entry that involve acquired immunity, innate immunity, and also HIV regulation. I'm going to show you three slides now which simply summarize these, and then talk a little bit about what they mean.

This is six genes that modify entry. I've talked about CCR5. There are others such as CCR2 and ligands, SDF, rantes, MCP, all of which have an effect on AIDS progression and have been published in pretty good journals and replicated in independent laboratories. These are genes that effect acquired immunity – that is, the ability to mount a humoral or cell mediated response. They are HLA homozygosity, the interaction of the natural killer cells in HLA, and then certain alleles of HLA that specifically regulate antigen presentation and epitope recognition that have been demonstrated directly to influence these cohorts and how well you do when you're infected with HIV. In addition to that, there are genes such as interferon gamma or cytokines like IL10 which influence AIDS progression, apobec, which is a molecule that is involved in interaction with HIV, the core HLA, a cancer, a regulatory transcription factor – all of these have SNP or mutation variations which up-regulate or down-regulate expression of these genes, which have like CCR5 delta 32, been linked to regulating quantitatively how rapidly a population progresses to AIDS or progresses to AIDS-defining conditions.

In addition to that, there is now a big interest in discovering the genes that influence why some people do better than others when they are treated with highly-active anti-retroviral therapy. The HAART therapy actually includes the idea of treating with three drugs so that mutational reversion is very rare, and they usually include a couple of nucleotide reverse transcriptase inhibitors two plus one not nucleotide reverse transcriptase inhibitors or other combinations.

But, unfortunately, it doesn't work for everybody. In fact, in the initial 18 months of a study population that we have looked at, the max, about 40% have succeeded and 60% fail. Within five years after changing the combinations and seeing what best, about 25% still fail. Some of that is due to individual compliance. They just don't take the drugs. But, much of it may not be. It may have a genetic explanation.

So, we guessed that there might be some genetic influences on three steps in HAART therapy. The first is failure to suppress the virus itself. The second is how slowly or rapidly a person goes on to get worse to develop AIDS. Third, the adverse events. We seem to be trading one disease, AIDS, in for hypertension and cardiac abnormalities, and lipid dystrophy and drug toxicity. The kinetics of this has a genetic component that influences it.

This is a slide that simply lists the names of some of the genes I've just shown you a few minutes ago. They are genes that other laboratories have implicated in influencing what happens with HAART. We investigated these, plus the other genes that we have already discovered, the 20 or so AIDS restriction genes, and tried to find out whether or not we detect any influence of these AIDS restriction genes on survival. The answer, in short, is we do, and I'm going to show you three quick slides that are survival slides that show you kind of what I mean.

This, for example, is the discrimination between people that basically have different genotypes for delta 32 after they have had HAART therapy and how well they do. It is good to be heterozygous – it slows down the rapid progression of AIDS in the presence of HAART therapy. This is another mutation in rantes – one of the ligands for CCR5 that basically shows a rapid progression in the rare allele. This is a slide which illustrates a different way of measuring survival, measuring the concentration of virus in hundreds of patients over the course of the time since they sero-converted, but before HAART therapy. This is starting at treatment and after HAART therapy. The delta 32 mutation shows a significant difference, although it is not a big deal.

Another way of looking at it is looking at the CD4 counts, which is the hallmark of AIDS development. In the absence of HAART, there is a difference in delta 32, and in the presence of HAART, there doesn't seem to be a very big difference, as indicated here.

For some of the other genes like rantees, the viral load seems to show a massive difference. These are just different ways at looking at how well a patient will do under these therapies.

In sum, these are the genes that we saw that had an effect in both HAART and in natural history before HAART. This is natural history before HAART. The red means highly significant and bad for you. You don't want to have this gene if you're on HAART. Green means it is good for you – it slows things down.

Viral suppression, progression to AIDS after HAART, CD4 slope – these are the summation of the results which show replication of some of these genes of being bad after HAART or good after HAART, as they were in the natural history studies and so basically the conclusion of this slide is not too surprising. But, one important point is that the replication of influences after HAART therapy means that ARGs continue to influence AIDS post-HAART – that means the virus is still replicating even in patients with it completely suppressed.

Two final points. The AIDS restriction genes – how important are they? This is a list of 20. They have different names. Some are dominant; some are recessive; they have different effects on HIV progression or infection. They have all been published, and many of them have been replicated in other laboratories.

Well, when epidemics vary in many ways, what are the guesses as to why there is a variation? Well, genetics of the virus. The genetics of the host. The patient's history. The non-genetic environmental influences, the poisons and the chemicals in nutrition, AIDS circumcision makes a difference, stochastic stuff – just bad luck for some people – or all of the above. Is it possible to quantify them? Is it possible to develop a yard stick so we know where we are in measuring genetic versus environmental influences?

Well, epidemiologists have a couple of things that they are used to talking about. One is relative risk, which is a measure of how strong the effect is. The second is attributable risk, which is when you go to the hospital and you get all the sick people, you saw what fraction of them are in there because they can carry a particular genotype. But, these numbers can be misleading for reasons that I don't have time to go into. A third, and I think more useful value, is something called explained fraction, which is to take all the epidemiological variance in AIDS survival – how much can we explain by the genotype of an individual, looking at all the AIDS restriction genes? In this we developed a method by mutual information theory using a small 2 x 2 contingency table. It is pretty simple. You can do it on a hand-held calculator.

What am I talking about here? These are the relative risks, the attributable risks, and the explained fraction for a number of AIDS restriction genes no the rate of progression. These are bunch of genes that are good for you – that you'd like to have if you got infected with HIV, CCR5 and so forth. These are bunch that are bad for you. What it does is it estimates the frequency of the genotype in the population, the relative risks, how much better off you are worse off. The attributable risks – what fraction of the patients in the hospital that are sick – you can explain by these. The explained fraction – which is how much of the overall variance can you explain.

Well, the totals are the bottom line, and the combined protective fraction is pretty high for the attributable risk – about 22% of the sick people we can explain by their composite genotype of one or more of these genes. But, the total variance – how much variability in the epidemic itself, taking everybody out three, is small – only about 10% of the variance we can explain. That means we have 90% more to explain. So, if we have a yardstick from 0 to 100 yards, we are only on the 10 yard line with the genes we know about. So, then 90% of the variation is not explained. So, how does this inference in form, the ability of our genotypes to inform clinical trials? Can we hand the genotypes of AIDS restriction genes to people doing clinical trials for vaccines and drugs and say, let's factor this into your equation so you can just subtract out the genetic noise? And, how well does the genotype imputed propensity index – this is the composite genotypic score, predict the time to develop AIDS?

Well, here is the answer. It is not very good. If we actually take the genotype of a bunch of patients and we predict how long it is going to take them to get AIDS, and then we actually measure how long it is going to take, this is the predicted. This is the observed for the same people. You get what looks like a circle. There is a slight regression, but it is tiny. That is what you'd expect since 90% of the variance we haven't explained this. We are only explaining 10%. So, this isn't ready for applications yet. So, comparing the predicted time to AIDS based upon the composite genotype, the actual time, we actually wind up with a disappointingly poor predictive value prognostic.

So, the short is only about 9% of the epidemiological variance is explained by the 17 genes. This is not enough for clinical trials.

What is the good news? Well, about 10% of . . . is actually quite a bit. If you do the same thing for smoking and lung cancer, we did that. Actually, smoking only explains about 10% of the variance too. So, these 20 genes that we have in the AIDS epidemic explains as much of the variance as smoking does in lung cancer, which we all know is a big deal.

In addition, genes with relatively modest influences can readily be discovered with these large cohorts. So, we think there may be some few more genes to discover and certainly other epidemiological things.

Now, my last five minutes will be developing into the future. We would like to take the haplotype map kind of idea and – let's go back. This is a summary of some of the milestones of the human genome project which epidemiologists want to use to discover new genes. One was in October of 2004. We had a human genome project which annotated the 20,000 odd genes in the human genome. Then, over the last several years, almost ten million single nucleotide variants have been annotated and a few months ago, the first one million snip level hap map was released, annotated in haplotype blocks. The average block size was about 14 kb in Caucasians. Beyond that, what is going on now is many species of mammals are being sequenced to identify regulatory reasons that are conserved through evolutionary inference.

Now, we wanted to see whether or not the haplotype-based analysis could be used and whether we could develop new tools, the subject of this conference, to help handle large amounts of genotyping data using the AIDS cohorts. So, what we did was we took 2,600 patients that were selected for highly informed clinical data and then we basically took eight known age-restriction genes and then we spaced snips across each of the genes going up 400 base pairs in one direction, and 400 in another. We built the haplotype structure around each gene, then we ran snip association tests for each snip using a real frequency, genotypes, dominant recessive, a number of different tests for AIDS outcomes. Total – 236 tests for each snip. They were basically broken down into infection effects, progression effects, age-defining conditions, and HAART therapy. What we wanted to do was evaluate the power of the causal or operative snip, and then what we call a proxy snips. These are the ones that are next to the operative snip, but are in linkages of this equilibrium, but track them in association – and then the haplotypes themselves.

These are the eight restriction genes we looked at – IL10, CCR2 and CCR5, chromosome . . . , rantees, SDF, and then two control regions: one region chromosome on #7 which had the CFTR regions which we didn't expect to effect AIDS, and then another region of chromosome #16 which was derived from one of these signatures of selection discovery panels.

Then we looked at each region for a number of things. This is a region of IL10 on chromosome #1. Basically, it is about 800 base pairs across, this is the linkage to the equilibrium pattern where the red indicates non-random linkage to equilibrium. These are the genes that are inside – IL10 is this one here. These are the snips that we interrogated. These are the haplotype blocks that are built by algorithms that allow us to know what the structure is. This is chromosome 3 – the same kind of idea – an LD scan here, the snips, the genes, chromosome CCR5 is here, CCR2 is here. These are the snips that were interrogated. Again, we are talking about hundreds of snips across all the genes, and these are the blocks with the number of haplotypes.

Now, a couple of new tools. First of all, . . . when you're talking about this number of snips and this number of tests and this number of genotypes, you're talking about 700,000 genotypes, you're talking about 200,000 combinations of tests. We need a better way to look at this other

than the way we did when we discovered CCR5. So, the first one that we developed was something that was based on a concept you've heard a lot about at this meeting – the affimetrix expression array. It is basically a heat plot where on one axis we list all the tests – the infection tests, the progression tests, the sequely or HAART test, and then on the other side we list the snips in the order at which they occur on the map. So, in all, the concept is based on the affimetrix express array chips.

So, what I'm going to show you is an expression array where the colors are indicating the P-value – how significant it is. The stronger the P-value, the better the color. The first one I'm going to show you is going to be the control region CFTR. Now, these are the snips that we assayed across the FTR and these are the 136 tests – chromosome 7. The orange means it is between .05 and .01. There are plenty of those by chance alone, and then there is a few .01. This is the background. This is what you expect when nothing is happening.

Now, let's go to chromosome #1 – IL10. In here, what you see is this operative snip, which is the red one, is this guy here and the blue ones are the proxy snips next to it. You can see the infection. You do see them infect, and if you blow it up, you can see which of the tests are actually firing and which of the data itself. Then we also look at progression and you see a bunch of highly significant tests here. Again, validating what we had hoped to see by simply viewing a whole bunch of tests and a whole bunch of snips at the same time.

Chromosome 3 was beautiful. This is where CCR5 is, and CCR5 promoter, and CCR2 – they are all linked closely together. As you can see, we see a very strong signal for infection here, a rather strong signal for a progression where you see lots of colors which means combinations of proxy snips, and different tests that are non-overlapping, non-independent. In addition, what is going on up here? This is not CCR5 or CCR2. It is a new region. They are showing us signals that we didn't know about before. That is basically what we expect in a genome scan.

If you compare CFTR, the chromosome 7 control region, with the CCR5 two regions, you can see this is what we want to see, define new genes, this is the background. That is really what the tool does – it gives us the chance to inspect it through a visual by eye.

We have another test which takes into account not only the P-value, but also the relative risk or the odds ratio – how much it is. What this does is it simply takes one snip and it looks at the 256 tests and it says, what is the lowest P-value we see for that test. Then, we take that P-value and we compare it to the next snip and the next snip. Then, we rank all 256 lowest snips and then we rank them.

Then, we do the same thing for the odds ratio divided by P. So, you take the highest odds ratio and divide it by the lowest P-value, and we rank it within one snip and then we rank all the snips compared to each other in the region and through all the other regions as well. So, these are five different kinds of ranks. One is snip the lowest P-value. The second is the number of tests where P-values are less than one. The third and fourth are odds ratio divided by P's, but at different significance levels. The fifth is just simply the average of the previous four.

What I'm going to show you is a plot which looks like the gene itself with all the snips along the baseline, but ranked for these five ranks, starting with the control region which is CFTR (cystic fibrosis). What you see here is the first, second, third, fourth, fifth rank. This is the gene itself, the gene region, 800 base pairs, about 50 snips ranked across. As you can see in this particular test which happens to be the fourth ranking, there is no dipping below 250. There is a couple here and there, but there is no consistency. This is a different analysis. This is for infection. This is for AIDS progression. You see a couple of things going on. This is a negative control. This is what you want to see in most regions.

Now, let's go to IL10 where there was a signal. Here what you can see is that there is a significant dip of the operative snip plus the proxy snips next to it for all five rankings. Bingo – that's a signal. That is what we want to see. When we look at the progression, it is also dramatic there. So, what you want to use is replication in the different kinds of rankings across that we can indicate. That is what we're looking for in this kind of test.

When we look at the chromosome 3 region, the one that was so bright on the . . . array, we also see a lot more action than we saw on the control region. There is a bunch of things around the

operative snip. They are showing it, including proxy snips, and there is one way out here that is in linkage to this equilibrium. For progression, this whole region is showing a big large signal.

So, the pilot conclusions, and I won't show you all the haplotypes, is the deoperative snips are detected pretty well. The proxy snips work about 80% of the time. And, the operative snip haplotypes actually work even if you removed the operative snip, they work 80% of the time. We do need better methods to quench the false-positive signal, but the prospects are pretty good for a full genome scan.

Final point – the signatures of recent selection in the human genome – there have been a bunch of papers out there looking for selection like delta 32 based upon different methods. Heterozygosity is something you expect to go down when a gene is selected because the genes will go to homozygosity and the markers around it will become homozygous.

FST – if you actually have two ethnic groups, say Asians versus Europeans, you would expect large differences if the gene were selected in Asia but not in Europe, and that would be illustrated by a big difference in the same snip in these two populations.

Linkages to equilibrium – take a look at the snip with respect to its adjacent markers. How long is the chunk? Remember CCR5 was about a centi-morgan long.

Then DSDN – remember the ratio of synonymous versus non-synonymous amino acid substitutions among polymorphic markers within coding regions. These are normal signals that people look at.

Up until this year there had been about a half-dozen really good studies that had found a number of regions across the human genome based on genethon, micro-satellites or snips or pEarlGen. These are different big data sets, the hap map data set, that had indicated this many regions – the green – that were actually signatures of selection. Of those, the answer was when you ask how many of the Hutley ones were represented by Carlson or Nielson, the answer was not very many. There wasn't a lot of agreement.

. . . lexic and our lab basically did it with a multi-point analysis, a moving window where he looked at five to 70 snips in a window and ratcheted up each chromosome for multiple things, and was able to increase replication of all these studies so that about 10-15% were replicated. I'm telling you this because one of the regions that we selected for a control for the . . . and ARG rank was one of these regions on chromosome 16 – a big region with a lot of LD with one big gene, a glutamate receptor gene called GRIN 2A.

Why am I telling you this? When we put it down on ARG array, we discovered not a background signal, but a real strong positive signal. Here is progression and down here in a region and here in infection. So, basically this was a negative control region originally selected for a signature selection and it was showing an effect as if it might have been selected by an ancient disease waiting for HIV to come along, just like delta 32. When you compare with chromosome 16 array to the chromosome 7 control region, you can see that this one looks colorful, this one does not, this is what we're looking for.

When we look at ARG rank, the same five rankings for chromosome 16 region, we find again affirmation with multiple snips that are associated in this region together, there also in progression, and there also in progression – two regions of the region.

So, what do we know about this gene? Not a lot. It is an NMPA receptor, a class of glutamate receptors. It is thought to underlie certain kinds of memory and it has an interaction with HIV tat and this is basically the final point. I'm almost done. Once the HIV genome enters the cytoplasm, it traverses micro-tubulus to get to the cellular nucleus.

So, can we scale up a snip hap . . . whole genome? I think we can. If we take this kind of an array, it actually looks like this. It is about 14 inches long. Here is another one that is 14 inches long. That is about 800 kb. If we scale up 2,000 kb, it is about a meter. So, a chromosome of the same thing would be 75 meters long, which is up and down this room three or four times. A whole genome would be 1,400 meters or about three-quarters of a mile. So, a genomics ARG array video highway, we could drive down it for three quarters of a mile and see all these signals.

Conclusions – argus can translate to patients. Entry and integration inhibitors are under development which have been stimulated by these discoveries. Explain fraction for the epidemic is small, but growing. Whole genome scans with available cohorts are feasible . . .

(Tape 13)

. . . these are folks from my laboratory, principal investigators. These are the cohort directors, the Chinese collaborators -- three of them are here. This is the folks in the laboratory – the principal investigators who have driven it, and this is the rest of the laboratory who does most of the work at our annual retreat.

The genome scan is a collaboration between LGD and the Brode Institute and is listed right here. We are looking forward to basically revealing all the age restriction genes we can find pretty soon.

Let me end with a quotation by Patty Stone. . and Richard Luger who said in the 1960's – we launched the Apollo program to put a man on the moon; in the 1990's, we came together to map the human genome. In the decades ahead, why shouldn't we demand a similarly urgent effort, this time an international one to stop this deadly scourge. I kind of agree.

Thank you for your attention.

Moderator – Thank you very much, Steve. Our next speaker will be Li Taisheng. Professor Li is at the Peking Union Hospital and will speak about clinical outcomes in advanced Chinese AIDS patients.

Li Taisheng, Peking Union Hospital

***Clinical outcomes and Immune reconstitution in advanced Chinese AIDS patients undergoing
12 months of highly active antiretroviral therapy***

Thank you Chairmen. Good afternoon. Thank for the organizers to give me this opportunity to give my presentation. My talk is clinical outcome and immune reconstitution . . . Chinese AIDS patients after one year after HAART.

(not transcribed)

Moderator – Thank you, Dr. Li. Our final speaker in this session is Lance Gable. Mr. Gable is a Senior Fellow at the Center for Law and Public’s Health at Georgetown University speaking on avian influenza and the risk of a pandemic.

**Lance Gable, Center for Law and the Public’s Health,
Georgetown University**

Avian Influenza: Preparing for and Responding to a Potential Human Pandemic

Thank you. I’m happy to see so many people have stuck around to the end of the day. It has been a great honor to attend this meeting. I’ve learned so much from all of you. I want to thank the meeting hosts and sponsors very much for inviting me to attend. I want to thank everyone who has been a part of this because it really demonstrates some amazingly innovative work that is being done in genomics.

I am not a scientist, so it sets me apart a little bit from the other speakers. My presentation will have no graphs. It will have almost no numbers. It will be mostly words and a few pictures. But, what I want to address is, using the example of avian flu and in talking about preparedness for and responding to a potential human pandemic, whether it comes from H5N1 or some other strain of the virus, I want to talk about how law, and to some extent, policy can inform the decisions that are made to respond and prepare for a pandemic. I want to also talk about how some of the scientific discoveries that we've been talking about for the past two days and how it will affect how those discoveries are used in a practical response effort.

So, I want to start off just talking a little bit about what I mean by a public health law. It is a fairly broad definition which I'll explain in a second. Then I'm going to talk about a number of different approaches that have been proposed to intervene in a pandemic, and talk about how some of these approaches are either effected by or can be facilitated by well-developed laws.

So, first, what is public health law? I'm going to define public health law as a two-part definition. The first is the legal powers and duties of government used primarily to assure the conditions for people to be healthy. So, for example, any type of law that assists the government or the people in the private sector to identify, prevent, and ameliorate risks of health in human populations.

There is a second component to public health law which is that it is not only laws that enable public health to occur, but this concept can include laws that limit the ability to conduct public health-related activities. So, there can be structural components that are put into place by law such as governmental structures. A good example of this in the United States, some public health powers are at the national government level and some are at the state government level, and I'll talk a little bit more about this in a few minutes.

Then there are also some laws that affect what individuals can and cannot do, and in some cases, might have privacy protections or something like that which would affect how public health laws can be implemented.

So, this two-part definition is actually very broad. It encompasses a whole range of different types of potential issues. So, what I'm talking about here is not only laws and policies that directly authorize the government, for example, to go and conduct disease surveillance or to take information and share it with other nations with regard to a pandemic outbreak. Or, for example, to impose a quarantine on people. It is also about other related types of provisions that might also impact the implementation of those types of powers.

I also want to point out that this idea of law spans many levels and obviously each country has their own set of laws that affect how they might be able to respond in a pandemic-type situation. There is also international law which applies across multiple countries, although international law is much less enforceable and a little harder to get a grasp on. Laws also exist at multiple levels within countries, in many cases.

I'm not going to actually spend too much time talking about pandemic influenza from a scientific perspective because that has been very well done already by some of the previous presenters. Dr. Monto and others have already given a great description of some of the risks and some of the potential problems that can be posed if an influenza pandemic, whether it is H5N1 or some other variant, gets into the human population and becomes transmissible human-to-human.

From a policy perspective, and from a perspective of how laws might affect this situation, there are multiple public health challenges that can arise during a pandemic. Some of them will be scientific, as we have been talking about for the past two days, but there are legal challenges. Are there appropriate powers, for example, to conduct all the types of activities, all of the interventions that you might want to during a pandemic. There are ethical considerations. There are political considerations, of course. And, there are financial considerations and many others.

So, in all of these types of interventions which I'm going to talk about in a minute, laws are an important tool, as are policy decisions and how the law is implemented. There may be some ethical considerations as well.

This is a map from the WHO which just shows that as of about a week ago, where the human

cases of H5N1 had been since 2003. You see most of them are concentrated in southeast Asia, but there are increasingly human cases spreading to other parts of the world. We know the infection in bird populations has continued to spread around the world as well due to some of these bird flyways that overlap across the globe.

So, with that as the background, I want to turn now and identify a couple of issues that I think are worth talking about and to talk about how medical countermeasures and public health interventions that can be used during a pandemic or to prepare for a pandemic can be thought about in the context of how law might affect these types of interventions.

I believe Dr. Monto referred to these similar types of interventions as therapeutic and non-therapeutic. I'm using the terminology of medical versus public health. But, it's the same essential thing. The medical countermeasures are things like vaccines, antiviral medications that can be used to treat patients or potentially as prophylaxis to prevent infections.

The public health interventions, or non-therapeutic interventions, are other techniques and strategies to try to track the disease, to try to restrict the spread between people without actually using any kind of medication.

Of these different interventions, each of these potentially has scientific, political and legal components. I'm going to focus on the legal components. So, first, with either type of medical countermeasure, whether we are talking about vaccines or antivirals, there are several challenges that need to be overcome to make sure that even if you pass the first test which is to have the good science in place, there is going to be time lag in developing a new vaccine for a novel strain. But, even once you've succeeded in developing your new countermeasures that you're going to try to distribute to the population, there are three other challenges that present themselves. You need to have an adequate supply, first of all, to treat the population or to circulate to the population. You need to be able to distribute it to people all across the world potentially. And, there are also issues related to ethically appropriate and allocation of that medication to make sure that the decisions being made are appropriate and who gets the

medication. Undoubtedly, there will not be enough for everyone. Even the most optimistic scenarios, we don't have the production capacity if it truly is a worldwide pandemic.

I want to talk for a second about funding for these types of medical countermeasures. At least in the United States, there was a proposal that President Bush put forward that calls for \$7.0 billion in funding for pandemic influenza preparedness. Of this money, about six billion of that is for medical countermeasures. That is divided \$4.7 billion for cell-based vaccine technology, and stockpiling experimental vaccines, \$1.4 billion for antiviral medication. So far, Congress has allocated \$3.8 billion – about \$3.4 billion of that is towards medical countermeasures.

Now, one of the things that strikes me as interesting about this decision-making and this planning is that the vast majority of the money in this proposal goes towards medical countermeasures. Very little of it goes towards any of the other types of interventions – such as strengthening surveillance, for example, trying to detect the disease at an early stage. It is an interesting decision. It is very reliant on the medical components and not really as much money is going towards the public health infrastructure.

Outside the United States, there is a great deal of variability in terms of what countries are doing to prepare. The funding obviously varies quite a lot, depending on the resources of the country. There have been some international efforts, actually, to try to raise money that the World Health Organization and other international bodies can use to try to have a global approach to preparedness for a pandemic. Just a few months ago, in January 2006, here in Beijing a whole group of countries came up with the Beijing Declaration which calls for not only a great deal of cooperation between countries in preparing for a pandemic, but also receive commitments from the attending countries of \$1.9 billion to towards these efforts.

Another important point about the Beijing Declaration is that it explicitly notes that the World Health Organization and the International Health Regulations, which are an international set of regulations that require countries to report certain information to the WHO and to work with the WHO for certain types of diseases, and the Beijing Declaration actually explicitly says the countries should be using this mechanism to report.

One of the main issues in terms of vaccine supply, and this is where some of the legal concerns can come into play. Vaccine supply has been inconsistent for seasonal flu and since there isn't capacity for seasonal flu, there certainly isn't capacity for a potential pandemic. In the United States, in 1967, there were 26 vaccine manufacturers that were licensed in the United States. This year, there were only four. That is not the vaccine manufacturers who make flu vaccines. That is all vaccines – only four in the United States. Worldwide, there are many less than there used to be as well.

So, you might ask the question well why are companies not making vaccines? There is a couple of theories about why this is the case. They are listed on the slide. One is the market forces are a disincentive to creating vaccines. Companies can't money on vaccines, so they are making other things instead – pharmaceutical companies are trying to go towards other products that may be more financially lucrative because vaccine purchases are inconsistent.

The other three suggestions on this slide here are actually directly related to the legal structure. There is the idea that regulatory compliance with making vaccines is a very high cost on the manufacturers. So, they are less inclined to make them. Every country has a regulatory system that regulates the quality of medications like vaccines. In the United States, it's the food and drug administration and the regulatory process can take a long time. It can actually end up deterring some companies. So, there have been some proposals to change the regulatory laws either to make them less stringent, especially in an emergency situation like a pandemic, or there have also been proposals to try to streamline some of the regulations across different countries. If you're going to be distributing this vaccine around the world, you have to meet the regulatory standards in all the countries.

Of course the regulatory standards are there for a reason – to make sure the vaccines are safe and they are effective. So, there obviously can't be too much of a dilution of these requirements. But, there have been a number of legal proposals out there that have suggested doing just that.

Another two legal issues, which are the bottom two on that previous slide but are talked about in more detail on this slide, are the ideas of liability protection and patent protection. So, liability protection – the concept of liability is a legal concept that basically says that if you are injured by someone, you have some kind of right to go after them to file a lawsuit if you are injured. This happens a lot in the healthcare system in many countries around the world and with respect to vaccines, there is a fear by a lot of vaccine manufacturers that especially if they are making a novel vaccine that hasn't been tested very much.

... would be sued by the injured parties. So, there have been some legal proposals, both in the United States and elsewhere, to prevent lawsuits against vaccine manufacturers who are manufacturing vaccines for pandemic flu.

From an ethical perspective and from the perspective of fairness, there is also the other side of the argument which is that people who are injured by these vaccines, should they have some ability to receive compensation for their injuries. In the United States there is a system for certain vaccines that allows just that. You aren't permitted to sue the vaccine manufacturer, but you are permitted to go to what is called the vaccine injury compensation program and make a claim that you were injured as a direct result of exposure to the vaccine and receive some kind of compensation.

So far, an avian influenza, vaccine is not on that list of vaccines covered under that program, but it is an interesting idea and it is a way that the legal system can be used to not only encourage companies to manufacture needed medications, but also to make sure that people who are injured might actually have some way to be compensated for their injuries.

The second issue here, patent protections – this is a very complex issue that I'm not going to get into too much detail today, but every country, or most countries, have patent laws that give exclusive rights to companies who create a certain product to market that product exclusively. It is meant to encourage innovation. Most vaccines are under some sort of patent and the worry is that vaccines, or other interventions, like flu, for example, the patent is held by Roche. Roche has been very reluctant to allow anyone else to use its patent to increase capacity. Roche itself

does not have the capacity to make enough tama flu for the entire world. Actually, in the China Daily yesterday, there was an article about the fact that Roche did license tama flu to another firm in China. I think that brings internationally three other firms outside of Roche themselves who are permitted to make tama flu. But, under international trade law, there actually is a way that if a country decides it needs a medication urgently that is under a patent held by a private country, the country can do what is called compulsory licensing. They can say we are going to make this medication because it is an emergency for our population. And, it is legitimate to break that patent in that context. The limitation on that is that countries that do not have the capacity to make their own medications cannot buy it from third party countries. So, if the scientific and the technological capacity isn't there to make the vaccines, that doesn't make a difference.

I want to talk just for a second about allocation and I'm not going to dwell on this at all. But, there are a lot of considerations about how – and this can be addressed by law or just by policy. But, questions of how you decide who gets vaccines or tama flu. If you have a limited amount of medication and you have a population that greatly exceeds this amount, how do you decide who in the population that gets the supplies. There are many approaches that you could use. You could, for example, use a public health approach which would say that you're just going to go after areas that are already affected and try and do something like ring vaccination or a targeted approach to areas that are directly affected.

There could be an approach where you would favor people who are in the scientific or healthcare communities or perhaps in the government and other critical infrastructure. Then there is also other considerations as well.

I know my time is short and I'm not going to talk about this now, but I would be happy to talk about this in the question and answer session if anyone is interested.

I want to highlight two more issues that relate to some of the non-therapeutic interventions. The first is the issue of surveillance and how countries can conduct the type of disease detection to

try to identify whether there has been human-to-human transmission of influenza that could cause a pandemic.

Surveillance is obviously one of the key tools to make sure that there is the ability to quickly respond and given the nature of how flu spreads, it is vital to have this early detection in order to have any chance at stopping a large outbreak.

So, two legal issues that arise with regard to surveillance is first, who has the authority to conduct surveillance. In most countries, there may be laws that allow the national government to collect data such as this. In the United States, it is actually a little bit different. The CDC has some authority to collect data, but usually the U.S. CDC gets its information from state health departments that collect the data. Also, universities and private companies are sometimes involved through contracts in collecting some of the surveillance data and there are many reporting laws as well which require certain diseases to be reportable to the government.

Currently in the United States, H5N1 influenza is not a reportable disease. So, even if – that is not to say it wouldn't be reported, but there is no law requiring it to be reported to the government.

Now, once this information is in the hands of the government, who has access to it? That is another important question that is usually addressed under the law. The law, in some cases, will put limits on who can actually access this information. There has recently been some debate about the influenza database that is being put together by the World Health Organization in that there is limited access and there are others who wanted to gain access to this data. This wasn't based on any kind of law. It was just the policy of the WHO. But, in the United States and in the EEU, there are data privacy laws that limit, to some extent, what personal information about health can be transmitted. Of course, both of these data protection statutes include exceptions for disease surveillance and for public health practice.

We are going to skip a couple of these. But, I want to spend the remainder of my time on issues of isolation and quarantine because these are the issues that are probably the most contentious

legal issues. The vast majority of countries have laws on their books that authorize the government to isolate or quarantine individuals who become sick in certain circumstances. Sometimes these powers to isolate and quarantine are very broad. Sometimes they are not as broad, but I guess the first point I want to make about this is that these two terms are often used interchangeably and they shouldn't be. Isolation is when a person who already has indications of an illness is separated from the general population so that they don't spread that on this further. Quarantine are people who have been exposed, but have not yet demonstrated any kind of detectable symptom and the idea behind quarantine is to keep that person separate from everyone until you can determine whether or not they are going to become sick.

There is also a third concept which is a group quarantine idea. It is sometimes referred to as and it is the idea that you would block off a whole section of a neighborhood or something like that to prevent people in that area from moving out and transmitting the disease to others.

Now, from a scientific perspective, there is a great deal of skepticism that quarantine will be effective at all in a flu pandemic, based upon the way that influenza spreads. So, the chances that these types of public health interventions will be effective has been questioned by many who know the ediology of influenza. However, it is very likely that many countries will try to use these powers, once there is an outbreak. The question from a legal perspective is who has these powers, when can they be exercised, are there any criteria that talk about how they could be exercised. So, it is just completely at the discretion of the President or the Prime Minister, or is it completely at the discretion of the leading health officer of the country. Or, are there some criteria that need to be met before people can be put under quarantine.

This is an issue at least in the United States, where the federal versus state government comes into play. Most quarantine power in the United States is at the state level. State health officers or the governors of the states actually have the power to authorize quarantine. It is sometimes delegated to officials in local health departments. But, the federal government only has quarantine power in very specific situations. People coming into the country at the borders, they can be quarantined by the federal government. People who are crossing state-to-state can be quarantined potentially by the federal government. But, people who are just in one place within

a state, the federal government does not have any kind of direct quarantine power. That has to be done at the state level. This is important because a lot of times there are plans proposed in the United States and elsewhere where political leaders will say, this is how we are going to conduct a quarantine. But, the reality is they might not have the legal power to do that, not to mention the fact that with any kind of restrictive measures like a quarantine, you have to take into consideration other factors about how can you do this successfully and how can you actually get people to comply. There are issues of safety and hygiene. There are issues of making sure the people who are put under quarantine have adequate medical care and necessities for life. If there is no food or water, people are not going to stay in quarantine. They are going to leave their houses or go to their jobs if they feel like they need the money to survive. That is an issue that most laws do not address and most policies do not address, which is how practically speaking can you implement this type of restrictive public health measure in a way that can allow it to be effective.

The final issue is just an issue about enforcement. Who enforces the quarantine. The people are not saying – if people are quarantined in their homes and they are staying in their homes, who actually is authorized to do that. Is it the military? Is it a police force? Is it some other entity? These are issues that all should be clarified ahead of time so there is not confusion if these powers do need to be used.

One final issue that I'm going to mention – there are other non-therapeutic interventions that could be used that may be more successful in a quarantine, but it is the flip side of the quarantine, which is to reduce opportunities for people to interact with each other. So, closing public places, canceling events, canceling schools for a time period potentially to delay the onset of the disease in whole sections of the population because they won't be interacting as much.

But, again, there are many legal questions that can be involved here. Who has the authority to close the schools? Who has the authority to make these decisions? In different countries, it is going to be different. Many times it will be the central government, but in other cases, it won't be. In the United States, for example, the schools are all done at the state level or sometimes even at the local level. So, just because one local school decides to close doesn't mean that the

others in that same state will necessarily close. So, there are a lot of things that are up in the air because of the variability of law.

I guess one of the things that I always try to tell people when I'm talking about these issues is that knowing the law ahead of time is important, and especially if the law is not set up in a way that allows for an effective response, thinking about maybe changing the law ahead of time is better. Laws that are changed in the midst of a crisis are usually not good laws.

There are also many issues that are related to international travel and border control. I know my time is running short, so I think I'll reserve this conversation for the question session if anyone wants to talk about some of the issues related to what countries can do to prevent people from entering into the country who may have the disease.

So, just to sum up, a well-developed legal structure can help with responding to a pandemic and these issues are important to think about ahead of time rather than after the fact.

Many of the scientific and logistical challenges that we have been talking about for the past few days, not only with avian influenza, but with any of the other diseases that we're talking about trying to work towards eradicating or reducing in the population, many of these also have legal components and it is good to keep that in mind.

The laws should be flexible enough to adapt to new threats. They shouldn't be rigid. Many of the old quarantine laws, for example, are directed at particular diseases. So, it will say you can quarantine for small pox, and that is not going to do anyone any good if there is any number of other diseases.

Finally, the laws should be as clear as possible to clearly authorize who has the power to engage in the different activities and what limits there are on those powers.

With that, I just want to thank you all again. If you have any questions, please send me an e-mail. This is my e-mail address and the website for the Law and the Public's Health. I also want

to thank two of my colleagues, Lauren . . . and Benjamin Berkman who helped with some of these ideas in this presentation. Thank you very much.

Moderator – Thank you very much for that. I think we have plenty of time for questions from the audience for all four of our speakers who I believe are here.

Question – This is a question for Dr. Li. Can you give us insight on what proportion of the HIV-infected patients are being treated with HAART therapy currently in China, and what are the restrictions that exist for broader use of HAART therapy?

Taisheng – The question is to the restrictions for the HIV in China? Please repeat your question.

Question – For patients that are infected with HIV, how commonly is HAART therapy being used in China and what are the restrictions to broad use of HAART therapy?

Taisheng – **(can't understand)**

Question – Can you give us the percentage – the number is just a . . . total number. What is the percentage of HIV positive persons?

Taisheng – About 20% of patients treated by HAART.

Question – I have one for Dr. O'Brien. As you expand your mapping to other regions of the genome, do you have plans to concentrate on certain regions like perhaps the “pharmaceutically attractable genome” to aid the pharmaceutical companies to get to the product faster?

O'Brien – Our major goal is not to get the pharmaceutical companies to the product faster. I guess our major goal is to discover as many verifiable gene regions that have translations of various sorts. We certainly are looking at the collection of pharmaceutically relevant genes involved in metabolism in our HAART population, and we are recruiting additional cohorts that

we're excited about, like the Vancouver cohort that has been sitting there for many years and has a thousand patients on treatment now.

So, the short-term goal is to really mount a full genome scan across the genome to get at the genes that we cannot guess at. Those are candidate genes – the ones that you described and we have put those down and they are in the queue to go on right now. But, I'd like to actually take advantage of the bioinformatics tools that have been developed now to scroll through the entire genome. So, that is what our major goal is, and hopefully it will include this group of genes as looked at from various ways.

Question – Steve, that raises an interesting point. The studies you and others have done are retrospective studies, and even under the best scenario where you identify another 20 ARGs that can account for 30% of the fraction of cases, how would you design a prospective study which is ultimately what you'd be interested in if you wanted to use these snips for any diagnostic purposes or to convert this to a prospective study would take an enormous period of time – much more time than any of us would want to devote to it.

O'Brien – I'm not sure I understand the question.

Question – In a retrospective study, you're identifying a series of snips that can explain why certain groups of people do better or worse or what have you. Before one would actually use that for any medical purpose, you'd want to do a prospective study.

O'Brien – If you're interested in diagnostics, yes, or in prognosis. The retrospective study can identify operative variance that quantitatively effects things in ways that we can then say that is useful for understanding the progression of the disease and gives us another avenue to develop therapies against, not by just simply using the gene, but by basically intervention – an intervention of some physiological phenomenon. That is one of the goals.

With respect to using the genotypes as useful for clinical trials, like for a new drug or for a prospective prediction of a patient at risk for something like that, it requires a pretty large

fraction of the explanation of the outcome to be a genetic. For example, 100% of cystic fibrosis is explained by mutations in the cystic fibrosis gene. So, finding that out is a very good prospective thing for prediction and counseling and things like that. But, if we're only going to explain 30% of the variance of AIDS with all the genes that are in the genome, then we are never going to be able to predict what is going to happen by just the genetics. We're going to have to know the other things that are non-genetic as well. So, I'm not so concerned about hitting that 100% target because I actually don't think we'll ever get there. As it is with any complex multifactorial disease, where there is a component that is genetic and a component that is not genetic, just categorizing them and understanding them – that information can then be translated to therapy development, perhaps to understanding the heterogeneity indexing or drug trials, but not always. In each case, they would be different. So, I'm not optimistic that we will get to the prospective stage at all in this particular disease because I don't think it is all genetic.

Question – I have a follow-up question to that. Given that these ARGs represent interactions with both the virus and drugs, have you tried building any of those issues into your model, your explained fraction model, maybe looking at the genotype of the virus at the same time that you're looking at the ARG snips?

O'Brien – We thought about that a lot, but we haven't actually figured out how to do it yet. The reason is that most of the virus genetic associations that we see with disease are almost anecdotal. That is, occasionally there will be a mutation of the neph gene or something that will cause it. But, even with the major clades, the argument that one is more or less virulent than the other is controversial at best. So, because we're talking about a virus here that has an immense opportunity for variance in the sense that it produces something on the order of 10 billion copies a day after a few weeks, and every second virus has a new single mutation and a cross of 9,000 base pairs or so. So it is a swarm, if you will, of billions of different variants that are produced in every patient every day. It is a recipe for resistance of just about anything that we want to get at.

So, trying to capture that as part of the variation, as a lot of people have tried it, and it has not been very helpful because of the fact that there is so much going on that we don't know how to interpret it very well – too much.

Question – I have a similar question. I want to ask, based on the other results you got, did you see any difference compared with the normal HIV patient with HIV carrier but long-lasting and no symptom patient? The reason I'm asking about this is because I think if we can better understand this issue, then maybe we can better to develop a drug and vaccine.

O'Brien – That is a very good question. We have paid very close attention to the long-term, non-progressors – those lucky patients who comprise about 1% of the infected population that avoid CD4 depletion for 20 years or longer. These patients have an elevated frequency of many of the protective AIDS restriction genes such as delta 32 and some of the HLA, B27, and B57 and some of the other AIDS restriction genes. But, as I mentioned, although the attributable risk of this group is pretty high, on the order of 20%, we can explain by their genotype, unfortunately the total explanation for why some people get long-term survival and other people progress rapidly like within six months. We have two patients, for example, who are homozygous for HLA B35 which is a bad thing to have, and both of them progressed to AIDS in six months. The reason that they do that is the B35 produces an epitope that monopolizes cytotoxic t-cell activity to the extent that the immune system is almost circled by a decoy, if you will. These patients progress to AIDS, as I said, in six months or less when they have that particular genotype. So, the answer is yes – the long-term progressors and the rapid progressors are very important parts of this continuum of survival that we see, and they are part of the analysis that allowed us to detect and quantify each one of these genes.

Do we know how much of the long term survivors we can explain by their genotype of the genes we know about? We're estimating it is less than 10%.

Question – This is question for Dr. O'Brien. There are three ways for HIV infection – blood, sexual and mother to children. If the people carried homozygous CTR5 delta 32, . . . prevention of HIV infection by three ways.

O'Brien – I did not understand the question – please say it again.

Question – My question is, if the people carried homozygous CTR5 delta 32, these can prevent . . . HIV infection in three ways – blood transmission, sexual transmission, and from the mother to kid transmission. Just . . . one way . . .

O'Brien – People that are delta 32 homozygous don't become infected regardless of how they are exposed. Does that answer your question? It doesn't matter whether it is a blood transmission or whether it is a sexual transmission or a contaminated blood factor or how it is. The only way that a homozygous delta 32 people become infected, and we have about five . . .

(Tape 14)

. . . original type specimen for HIV. They had psoriasis and sores on their hands. They concentrated the virus. They became infected with HTLD 3B which is HIV X4 tropic virus became infected. Their CD4 cells were depleted and they developed AIDS. This actually was probably the best example for why HIV fulfills cokes postulates because it showed that it transmitted and caused the disease. That is important because there is a conspiracy that HIV doesn't cause AIDS – that it continues to surface every year

Question – I have a question for Lance. Could you evaluate, on a scale from maybe 1 to 10, how prepared you think the U.S. is in the event of a human-human transmission outbreak of H5N1, highlighting specifically the points you mentioned related to quarantine, and general overall response. As you mentioned, the overwhelming amount of funding is going to medical preparedness and how you think that is going to influence the effectiveness of any response.

Gable – I think it is a good question. It is really hard to estimate. It is one of those things that think if I had to say on a 1 to 10, I would say it is certainly right now probably anywhere from three to five. It seems to be getting better because there has been a lot of attention paid to this potential threat in the past few months. But, one thing that I always – at least for the past few years that I have been looking at some of the different legal approaches and policy approaches to preparing for different potential disease threats, I think that when so much attention is paid to a particularly disease, and in this case we're talking about the potential for a flu pandemic, and

also when so much attention and so much of the financing goes to specific medical interventions, or some kind of specific approach to target just one disease, I think that is not as effective as when you put the money toward more broad-based, structural preparedness types of initiatives. So, for example, building a really robust public health infrastructure that will be able to respond both to a flu pandemic and all of these other many types of disease threats that are out there, I think is probably the way that we will become the most prepared.

I think that at least with the focus on the medical interventions, they can be very effective and very important. The problem is, at least with one component of that, the vaccines, there is going to be a considerable lag time. If we can manage to make the breakthroughs necessary to do a cell-based vaccine, maybe that will solve some of that problem. But, I think right now, having the vast majority of the resources going to that is not putting the United States or others that are adopting that approach, in a great position because there is going to be far too many people if there truly is a large-scale outbreak for those medications to actually reach.

Moderator – We have time for one more question.

Question – I have a question for Dr. Li. My question is, I take care of patients who are primarily Caucasian in San Francisco. My question is, have you noticed any difference in the response to HAART in Chinese patients versus say a comparable cohort of Caucasian studies. There have been many studies on individual developed therapy. The second question I have is have you noticed side-effects from HAART therapy and is there any difference, again, between the side effects and potential side effects seen in the Caucasian population?

Taisheng – **(cannot understand)**

Moderator – Please join me in thanking all the speakers one more time. I believe we now have tea, followed by posters.