Introduction of Keynote Speaker

Dr. Rick Kittles

Co-Chair, Conference Scientific Program Committee

I am charged today with introducing Dr. Carpten, whom I have known since 1997, a good friend and colleague. Dr. Carpten attended Lane College, receiving a degree in biology, then attended Ohio State University and received a PhD in molecular biology in 1993. He was one of the first African-American males to achieve the PhD at Ohio State University. I spent some time at Ohio State. I remember when going to work, passing by the department, some faculty would remind me; I would say yes, I know John. They would say yes, he was a great student. No, I know him; I know he is a great scientist.

I met John in 1997 when I had just finished my PhD. I was at George Washington University. I was doing work at NIH, and I saw an ad for a study coordinator for a study on African-American hereditary prostate cancer, and it was a national network. There are several individuals in this room who were part of that network, which was historic because it is unlikely that something like that will happen again where scientists, urologists, radiation oncologists, nurses, students of color, were all involved from top to bottom in a study focused on the African-American problem of prostate cancer. I was blessed to be a part of that. I remember working with John, who was a tireless researcher.

I really look forward to the long conversations that we have on the phone. Normally, they are late at night because I am on the other side of the country: he is in Arizona, and he would call in the evening and it would be late where I am at, [laughter] and we would talk about science, but then we would talk about other things; he would rejuvenate me and motivate me. This guy is spectacular. He stays on the cutting edge of genomics, cancer genomics in particular, and technology.

Keynote Address: African-American Hereditary Prostate Cancer Study Network: Where Are We Now?

John Carpten, PhD

Director, Integrated Cancer Genomics Division, Translational Genomics

It is fun to talk about the faculty at Ohio State because it did not seem they felt that way about me when I was there since I was on academic probation almost the entire time. [laughter] I was still the second person to finish in my class. [laughter] [applause] It was an awesome experience; I would not change it for the world and I learned a lot during those days.

I am glad I was asked to speak on this topic. This was an amazing effort and an amazing endeavor, and is one of those projects that I think will always stay hidden from the

mainstream scientific community for whatever reason. I don't think it was ever given the credits and accolades that it deserved, but for those who were a part of it, including Francis Collins, current Director of the NIH, I think for many of us, this study will go down in history as one of the most outstanding achievements in genetic research. I will go through the project and give some of the background and history, talk about the results, then finish up discussing what we have been able to accomplish over the time in which we actually had funding. When I got to TGen in 2003, I used pretty much my startup money and my own discretionary funds to finish this because our peers did not seem to feel that Ike's [phonetic] first two versions and my last version were up-to-par for funding, but that is okay; we got it done anyway. I will give some background on genomics and genetics, then come back to some of these concepts so you will have somewhat of an understanding of how particular ones fit into the study overall.

In genetics, we tend to deal with this central dogma, a very simple concept: we start out with DNA, which is transcribed into RNA, then translated into proteins. The proteins provide function and structure for cells to function normally, which leads to normal tissue development. DNA is made up of a series of building blocks, or nucleotides, of which there are four: A, T, C, and G, essentially linked together by phosphate bonds. They are constructed into two complementary strands: A always binds to T, and C always binds to G, and this leads to the famed double helix.

DNA in cells is organized in the chromosomes and into genes; there are about 30,000-40,000 genes in the human genome. Taking the whole idea of central dogma, if you start out with an abnormal gene, you end up with an abnormal protein, which generally leads to an abnormal cell and tissue, which ultimately leads to disease. Keep that in mind: bad DNA, bad protein, bad cells, bad tissue, disease.

We use a series of technologies, for instance, DNA sequencing, to identify these changes that can occur from individual to individual. We can track nucleotides and how they are inherited from parent to child. If we map these across the genome, we can identify regions and how these particular changes might affect a protein. This cartoon shows a polymorphism: there is a difference at the DNA level, but it results in the same protein. In many cases, we are hunting for situations where a change, in this instance, C to T, causes a dramatic change in protein sequence; it is those types of changes or mutations that we search for since in many cases, these are the types of changes that confer a disease in humans.

We use these genetic markers as landmarks that are passed from parent to child, and we can track the inheritance across families; this can reveal what DNA sequence is only in the affected and not in the unaffected individuals. If this was a family with prostate cancer, the circles are female, the squares are males, and if the box is blackened in, it would suggest that these individuals have been diagnosed with prostate cancer, but these two sons have not. So of the five sons, three have prostate cancer.

We can track the inheritance of genetic markers to get a picture of different chromosomes. This slide suggests that perhaps a gene is in that region which confers prostate cancer predisposition in the men in this family. If we look across a large set of families and repeatedly find that same region, this suggests presence of a gene in the mutated region that is tracked through the family, resulting in prostate cancer.

This inheritance is not just with prostate cancer; we see this in many diseases. These types of linkage studies by the early 1990s had identified a number of inherited mutated genes that caused diseases such as cystic fibrosis, Duchenne muscular dystrophy, as well as a series of hereditary cancer: breast, colon, and melanoma. Individuals with a mutation in their germ line, a mutation that is tracking through their family, those families have clusters of disease. Jeff Tritt [phonetic] then was working with Pat Walsh [phonetic] and Bill Isaacs [phonetic] at Johns Hopkins to identify a hereditary prostate cancer gene.

About 75% of prostate cancer is sporadic in nature; these are men who do not have a family history of disease but have the same types of characteristics as other men with prostate cancer. Yet, about 20% of men with prostate cancer have familial prostate cancer, meaning they have at least one family member, and about 5% of men have what is considered hereditary prostate cancer, meaning there are multiple individuals that have been diagnosed with prostate cancer in the family. Twin studies show that of all the cancers, prostate cancer had the highest heritability, meaning that there were more times where you saw two brothers with prostate cancer than you did other cancers. When taken together, these data would suggest that there are likely hereditary prostate cancer genes, meaning genes that are mutated, the mutated copy is passed along to the offspring and predisposed prostate cancer in those individuals.

We began to undertake these studies with colleagues at Johns Hopkins. As background, prostate cancer is the most common male malignancy in the world; an estimated 192,000 cases were diagnosed last year; about 28,000 men, or about 15% of those men, died of their disease. Prostate cancer is the most common cancer in African-American men, accounting for roughly 40% of cancer in this population. Autopsy studies show that prostate cancer in African-Americans manifest at a younger age and is more biologically aggressive regardless of socioeconomics, access to health care, and other economic factors.

The two most significant risk factors for prostate cancer are ethnicity, being African-American, and family history. When we look at race and ethnicity, several studies have shown that if you look at prostate cancer five-year relative survival rates, African-American men tend to do worse compared to our Caucasian counterparts, and overall, we just tend to do worse. If we look at incidence and death rates, we see that the incidence rate is almost twofold higher in African-American as compared to Caucasian, and death rates are nearly three times as high in African-Americans as Caucasians.

Family history is a very significant risk factor for developing the disease. Being of African-American descent and having a family history of disease exacts a heavy burden, and questions exist on whether these risk factors might be associated with socioeconomics, especially the aspect of African-American. There are a number of nonbiological factors that are sure to play some role in the disparities that we see, including socioeconomic, access, and reimbursement issues; culture and stigmatisms; diet; and environment. Any of these aspects can confer some type of negative factor towards influencing disparities seen in underrepresented populations like African-Americans. It is more difficult to associate these factors with familial clustering.

There were a number of studies looking at odds ratios, or the effect of family history on prostate cancer; a couple of these studies looked at both European-American and African-American men. This study shows that the odds ratios for having a first degree relative for African-American is over three, and for European-Americans is around two. This other study showed a very similar odds ratio when looking at African-Americans, but importantly, they showed that this increased risk was unchanged when they made statistical adjustments for socioeconomic status, education, income, and marital status.

All this information taken together provided an opportunity for us to understand the role of biology in these high risk prostate cancer families of African-American descent. When I got to the Genome Institute, I had gone there to work with Francis Collins and study the genetics of diabetes. I grew up in Mississippi and I rarely heard about cancer growing up as a child, but what I heard all the time was sweet diabetes, sugar diabetes, and strokes and heart disease. Diabetes and heart disease are what took my father out, so I had this passion that I wanted to study diabetes. I knew that Francis was starting the Genome Institute, and they invited me to interview. I wanted to work with Francis on diabetes, and when I went in the conference room to talk to Francis, he said, well, John, I will be honest with you: you are qualified, love to have you working on this, but I already have five post docs working on this project, and there is no way that I could give you a piece of the project that could be yours, which is what you need as a post doc, something that you can take forward sort of as yours. That turned out to be the best advice I ever got in my career because Jeff Tritt was like, yes, I have a perfect project for you to work on, who was working with Bill Isaacs and Patrick Walsh at Johns Hopkins to study hereditary prostate cancer. Jeff Tritt said, we are going to do all the genotyping here, we are going to collaborate with these guys; it is going to be really exciting. I said awesome.

We started working on this project in 1993-1994, and one day I was sitting in the lab with Jeff Smith [phonetic], the other post doc, sitting at our computers, analyzing data and looking at pedigrees, and I had this come-to-Jesus moment. [laughter] For whatever reason, this light just went off in my head; it was like, out of these 100 families, these pedigrees you are looking at, how many are African-American, right? This question just came out of nowhere. I looked at Jeff and said, Jeff, how many of these families are African-American? He responded, I don't know; why don't you ask Bill?

I immediately got on the phone; this was 1994 and we were just starting to get into email at the time, and did not have texts at the time. I left Bill a message and he returned the call and said, yes, well, John, you know, I think two of them are African-American. I said, wow, and was really understanding a couple of points: one, understanding the fact that prostate cancer was exacting a heavier burden in African-Americans; two, knowing the location of Johns Hopkins after having gone to Hopkins a couple of times, right in the middle of the 'hood. [laughter] Bill and I started a dialogue, and he said, well, I am going to bring in Sally Isaacs [phonetic], the nurse coordinator, and we will have a conference call and talk about it. Sally said, well, John, to be honest, we have had lots of African-American men come through, a lot of family history, but for whatever reason, they just did not trust us, they did not want to enroll in the study, they just had this thing about research; for them it was, look, treat my disease and leave me alone. What can you do to help my disease? I am not trying to call my brothers, I am not trying to bring my wife and the kids; we don't want to be giving away blood samples and DNA.

And so, being my Mom's son and my Dad's son, that all of a sudden became a challenge, an opportunity to do something new, different, innovative, but more importantly, do something for my community. As we talked to other individuals across the nation, including people like Cathy Cooney [phonetic] at the University of Michigan, Janet Stanford [phonetic] out in Seattle, and all the other big groups who were doing hereditary prostate cancer work, we found out that only about 2%, maybe 3% of the families that were being studied were African-American, even though African-Americans were twice as likely to be diagnosed with the disease. The same things came up repeatedly: difficulties in ascertaining families, no targeted recruitment, distrust in medical research. There seemed to be an issue with education about genetic research, not really understanding what it was we were trying to do. Of course, due to the high rates of prostate cancer, there was an obvious need to assess the population.

This opportunity led to the development of a study team, and from my standpoint, it required a special team of clinicians and scientists, a group that could break down the barriers of distrust and a group that could design and execute a state-of-the-art genomics research study to understand population genetics, genomic technology, and to utilize the complex statistical analysis packages required for such studies. From that, we were able to develop the African-American Hereditary Study Network. Georgia Dunsten [phonetic] came to meet with me after I spoke with Jeff and Francis and told them I really, really wanted to do this. After I met with Georgia, the first thing I knew I needed to do was to get my hands on the right group of clinicians.

And so, we reached out to the urology group from the National Medical Association. I remember talking to Francis, and I called Ike; Ike did not know me from Adam. I think Ike and Georgia went to college together. They flew into National Airport, and we met in Crystal City. I don't even think they left the airport; I think we met at the airport because they were like, okay, we will fly in and listen to what you have got to say, then we have got to go, because I was a post doc, right? Who am I? I was nobody. Actually, I had to beg Francis to go with me to try to bring someone who had some credibility.

When we got there, it was big love, and I talked to Ike and they were really frustrated about their issues with not being able to obtain funding from the NIH. They were right in the middle of the PSA wars trying to show the benefit of PSA screening and how they could help men and how all of their grants were being shot down. So they came in, they were battle-tested, they were mean, they were ready to go, and they said, we will help you. And so, Ike, and Brian was there, and so, we were able to form this incredible clinical team, which included Ike Powell [phonetic] in Detroit, James Bennett [phonetic] in Atlanta, Curtis Pettaway in Houston, Shia Gotu [phonetic] at Howard University Hospital, Sally Weinrig [phonetic] who was a PhD nurse who headed up the group in rural South Carolina, Jerry Hoke [phonetic] and Brian Stone in Harlem Hospital, Terry Mason [phonetic], and Trini Vassan [phonetic] Vejercomar [phonetic] who were both in Chicago at the time, and just an amazing cadre of clinicians and urologists, then Joan Bailey Wilson [phonetic] and Agnes Baffu [phonetic], Bonnie Agnes [phonetic] was a PhD in statistical genetics at Fox Chase Cancer Center, and so, Agnes joined the team. So you can see this team we pulled together was just an incredible team. Georgia Dunsten was the head of the coordinating center. Again, we hired at the time a young Rick Kittles fresh out of graduate school, and we also brought in an expert in medical and

genetic ethics, Charmayne Royal [phonetic], to help us with a lot of the community aspects that were important for this particular study.

Targeted recruitment of African-Americans: this study was funded by the National Center on Minority Health and Health Disparities, which was directed by Dr. John Ruffin [phonetic]. This study will always go down as a model for genetic research in minority populations. Specific AIM1 [phonetic] was to identify and recruit African-American families for participation, Specific AIM2 was to determine whether known regions of hereditary cancer such as HPC1 were contributing to familial prostate cancer in African-Americans, and Specific AIM3 to search for novel regions in genes that may contribute to familial prostate cancer in African-Americans. Seven collaborative recruitment centers were: Chicago, Detroit, New York, Washington, D.C., rural South Carolina, Atlanta, and Houston.

The inclusion criteria were four or more affected men, so there had to be at least four cases of prostate cancer in the family; average age at diagnosis in the family was less than 65 years; and at least three of the cases were willing to provide a blood sample for DNA. We were able to publish on the recruitment experience, showing that a physician referral was by far the most important method for collecting these families; since we had the right team of urologists with us, we were able to have successful recruitment.

Importantly, we wanted to get past the whole race issue; I think we were among the first to move in this space. I have to contribute this primarily to having Rick onboard and being adamant that we could not have men come in and self-identify as African-American and not really understand what that meant. Rick looked at the average percent ancestry in the different demographic populations named earlier. We genotyped our family members for these markers to correct for the European ad mixture issues. We recruited 108 families and were able to get full collection of DNA on over 80 families. This is one family from Houston: 11 men with prostate cancer in two generations; this man had sons in two relationships and sons with prostate cancer on both sides. These are the types of heavily weighted prostate cancer families that we recruited into our study.

We had several regions of the genome that had been linked to prostate cancer before we started, one of which was HPC1 which I worked on as a post doc, and another region, HPC2. We showed that none of the alleles at HPC1 or HPC2 were associated with familial prostate cancer, so when we screened family members, we did not see an association; we saw some light evidence of association here in the sporadics but nothing in the familial cases, suggesting that those two genes were likely not influencing hereditary disease in our families. We then looked across the entire genome for new regions that might be associated with hereditary prostate cancer in our families. Our findings suggested that there are about 100:1 odds that there is a gene here on chromosome 17 that is contributing to prostate cancer in our families. We went on to do more sophisticated analysis and were able to reach 1000:1 odds, which is very significant and suggests that there is a gene somewhere in this region on human chromosome 17 that is conferring risk of hereditary prostate cancer in our African-American families.

We added additional markers around that region on chromosome 17; we were able to not only show that the regions remained significant with the new markers, but in some cases, the LOD scores actually went up. Thus, we have very strong evidence that there is a

gene here conferring risk of prostate cancer in our cohort. We are working on writing this work up based on a combined analysis with additional African-American families from the International Consortium for Prostate Cancer Genetics. We have another peak on chromosome X which we will be following up. More recently, we have started using these new sequencing technologies that will let us sequence a human genome in less than a month for about $20,000; I have ten of these machines in my laboratory at TGen. We have sequenced the entire set of human exons in those eight individuals that are linked to chromosome 17, and we are going to do another eight that we are working on with another one of our collaborators at the University of Michigan to see if we can identify these mutations that are conferring risk in our families.

A number of genes that are associated with hereditary cancer are associated with somatically mutated genes. Somatic refers to mutations that are tumor acquired. Tumors change; the genomes change. The tumor cells acquire mutations in DNA. Even though some women are born with mutations in BRCA1, the way a breast cell becomes cancerous is that that cell actually undergoes another hit around that gene in the other chromosome that makes you have complete inactivation, or you completely lose the gene. These genes, like BRCA1, BRCA2, P53, P16, and APC, actually are mutated in cancer cells as well, so not only can you inherit a mutation, but the cancer cells themselves become mutated at these loci.

One question I asked was, are there genes that are known to be somatically mutated in prostate cancer that actually might confer risk of prostate cancer? One such gene is the EPHB2 tyrosine kinase receptor. My group found mutations in this gene in prostate tumors, and one mutation called K1019X truncates the protein, making the protein shorter. But when we screened 200 normal Caucasian DNA samples, mutation was seen in 1.5%, suggesting not necessarily a somatic mutation but that this nonsense mutation is present in people's germ line. People are born with this particular genetic change.

We started asking other questions. We showed that if you put a normal copy of EPHB2 into these prostate cancer cells that do not have any EPHB2, you can stop the cells from growing. Taking normal prostate cancer cells in a Petri dish, if we inject gene EPHB2 into these cells, we can see growth almost stopped completely, suggesting this is a tumor suppressor gene, suppressing growth of tumor cells, specifically prostate cancer cells, although work has also been done in colon cancer.

Thus, EPHB2 is a strong candidate prostate cancer tumor suppressor gene. Rick and I decided to screen all of the exons for this gene in our African-American hereditary cases to ask the questions, are there mutations in our hereditary cases in EPHB2 and might these mutations confer risk in our families? We were able to publish this research. We screened one individual from 72 families; Rick also was able to collect a set of 180 sporadic prostate cancer cases. We also had about 320 African-American controls, and these were men who did not have prostate cancer or BPH but were age-matched. We identified a series of mutations in these individuals and found the K1019X mutation that we had found earlier; it was present in Caucasians. We saw it in 15% of our African-American cases. Going back to our original paper, it was in only 1.5% in European Americans. This would suggest that the mutation is in ad mixture disequilibrium, meaning it is more common in Africans than Caucasians.

In looking at our African-American hereditary cases, our sporadic cases, and healthy controls, it was 15% in the hereditary cases but only 6% in sporadic and 5% in normal African-American controls. The mutation is three times more common in African-Americans; the mutation conferred risk of prostate cancer with an odds ratio of three. On family history, having one affected family member increases your risk twofold; having this mutation increases your risk threefold. This was a significant finding.

The mutation presents an interesting dilemma. A number of individuals are using these GWAS, genomewide association studies, to look for risk factors for different types of cancer. There is a large GWAS currently ongoing in African-American prostate cancer cases, and they use these microarrays with a million SNPs on them, with a SNP every 3000 bases or so. Using that type of technology, would we actually find this mutation? The chances are probably no. If we look at the human HAPMAT project, the mutation resides right here, and you can see that there are three SNPs in this region that result in a haplotype in the West African HAPMAT population but not in the European population.

We feel that this gene is a strong tumor suppressor. Rick and I were actually able to get an RO1 funded to follow-up on this study with AIM1 being to determine whether or not common variation in EPHB2 is associated with risk in sporadic disease. We just finished that, and that paper was submitted I think two days ago. The second AIM was to determine whether or not that particular mutation, K1019X, is functional: does it actually have a real functional consequence on the protein itself?

I had as a summer intern student, Hidar Hussein [phonetic], who worked with my post doc; we undertook this particular study. We generated a construct with a mutation in it, we put the construct into D145 cells, and we asked the question, can the mutated form of EPHB2 slow down or stop D145 cells from growing like the wild type version does? We were able to introduce the mutation into the construct; here is the wild type version and here is the mutated version. We then built expression vectors, and we used a Linte [phonetic] vital system which incorporates the vector into the host genome. We were able to show that we got the construct into the cells, and that the gene is expressed; both wild type and mutated versions were expressed in cells following transfection.

Looking at the empty vector, the cancer cells are growing fast. If you look at the wild type EPHB2, you can see it slows the cells down from growing; we had shown that earlier. Looking at K1019X, they are growing like you did not put anything in these at all, suggesting that that particular mutation is conferring a strong functional consequence on EPHB2. If that version of EPHB2 is in you, and it is mutated, that particular copy of EPHB2 is nonfunctional, meaning that if you have a prostate cell with that mutation, and that prostate cell undergoes a second hit and you lose the other copy of EPHB2, that cell can then begin to grow like a prostate cancer cell, a very important finding.

We demonstrated that K1019X confers a negative functional consequence; the mutation does not suppress growth of D145 such as wild type EPHB2. This study supports the notion that this mutation is an actual functional variant, and is important in African-American men with hereditary prostate cancer.

In closing, I hope I was able to give you an update on where we are with the study and some of the accomplishments. Unless you plan to live to 125, you will be fine; PSA

cannot give us that information now. What it will say is there is something going wrong; again, it is better safe than sorry.

Male Participant

Just one point: you do not have to go all the way to Africa. [break in audio] —percent mutations of BRCA1 and BRCA2. The island is the Bahamas. They have published that, our medical oncologists along with folks from the University of Miami, and presented it a year ago. As I am listening to you, I wonder how can we get that in the Caribbean, in the Bahamas? How can I get that linked to the University of the West Indies? You cannot just keep that here in the U.S.

Dr. Carpten

We have a very active program going on in Barbados, Clare Bonker [phonetic] has one ongoing in Trinidad and Tobago, and I know Rick was collaborating with people in Jamaica. I do not know about the Bahamas. You make a great point: in many genetic instances, we do want to look for, and I will say this delicately, homogeneous populations or groups of people where the gene pool is fairly similar. Those populations tend to provide a unique opportunity for genetic research. We did some rudimentary studies with BRCA1 and BRCA2 in Barbados, and did not see anything major jump out at us. We are going to continue those studies looking at familial clustering; some of the populations are very small.

Dr. Odedina

Thank you, Dr. Carpten, for a great presentation. Housekeeping announcements: the keynote speaker was sponsored by Mayo Clinic, so thank you, Mayo Clinic. Also, in the true spirit of research, we do have a research group from University of Florida who are looking at some studies in men in general, so please take some time to complete the survey and participate in ongoing studies.