



PROSTATE CANCER
FOUNDATION

Prostate Cancer Genes

Begin to Tell
Their Story

It's easy to wax nostalgic for the innocence of the late 1990s, a time of surplus budgets, technology stock bubbles, ever-expanding home ownership, and when finalizing a map of the human genome held the promise of quick solutions to age-old diseases. Yet the third millennial discovery that things are not as simple as we'd imagined at that end of an era moment in time, allows us to get on with the business of finding solutions unencumbered by false hopes. As distressing as it was to realize, in the latter case, that the mind-boggling complexity of human genetics meant not only would scientists have to find more than one "bad" gene for most diseases, they'd also have to trace the byzantine pathways of interconnectedness between genes, find co-factor proteins and molecules that affect genes, catalogue the epigenetic chemicals that sit atop genes regulating their behavior, etc.—all in order to fully contain the torrent of factors that initiate and sustain the disease process—as distressing as this realization was, it also put researchers an important step closer to curing disease.

While we now know that the vast majority of cures will not come all neatly packaged with a single-gene bow and that the very definition of cure for many diseases will be chronic drug management, we also are on a more realistic path to more realistic cures. Indeed, the opening chapters of the 21st century have been good ones thus far for prostate cancer genetic discovery and the precisely targeted drugs and diagnostic tests that often follow on the heels of such discovery. Recently, the director of the federal Food and Drug Administration (FDA) cancer drugs office, Dr. Richard Pazdur—known for his caution in approval of new drugs—crowded to the *Associated Press* that he was seeing "major advances" against prostate cancer, especially in terms of precision treatments that are often guided by genetic discovery.

This year also saw the discovery of the first gene, *HOXB13*, specific to hereditary prostate cancer risk and the FDA approval of a urine test for prostate cancer that measures the products of the *PCA3* gene that's hyperactive in prostate tumors and predictive of biopsy



PCF-funded researchers at the University of Michigan helped identify the 27+ genotype varieties of prostate cancer. Some are indolent, or non-life-threatening, while others are very aggressive.

results. And, researchers are identifying tiny variations in the individual letters of genetic code—SNPs, or single nucleotide polymorphisms—that are linked to prostate cancer at a blistering pace. Ethnic genetic variations are also coming to light with the finding in 2005 that over 50 percent of all Caucasian prostate cancer patients carry an abnormal fusion of two genes and the more recent discovery by Chinese researchers of several other aberrant gene fusions in Asian men with prostate cancer. After a long wait, it's starting to look like prostate cancer genes may finally be willing to write their memoir.

Coaxing prostate cancer genes to give up their ghost began in earnest in the early 1990s when Dr. Patrick Walsh, at Johns Hopkins University School of Medicine, reported that a subset of men with prostate cancer appeared to have an inherited form of the disease. While it's true that every single cancer is a genetic malfunction event that results in cells-gone-wild growth, only some cancers result from genes that are inherited from our parents. The vast majority of cancers, including prostate, arise sporadically and are likely to occur later in life. But for men under the age of 55 diagnosed with prostate cancer, hereditary prostate cancer susceptibility genes may account for half of all cases. And twin studies show that up to 42 percent of the risk for developing any type of prostate cancer may be inherited.

Separating the Genetic Wheat from the Chaff

Because a government task force recently recommended against routine prostate cancer screening due to the risk of over diagnosis of non-lethal cancers (currently one in six men are diagnosed), it may be easy to forget that this cancer kills approximately 30,000 men in the U.S. each year, making it the second most common cancer-related cause of death in men. And, because prostate cancer can be slow growing and non-life threatening, or aggressive and lethal, and because certain lethal forms have been linked to inherited risk, prostate cancer gene hunters are especially invested in finding the genetic drivers of aggressive forms of the disease.

"There is most definitely an inherited predisposition for prostate cancer," says Dr. William B. Isaacs, professor of urology and oncology at Johns Hopkins University School of Medicine and the principal investigator on the International Consortium for Prostate Cancer Genetics (ICPCG)—a group formed in 1996 that now studies familial prostate cancer in over 2,000 distinct families. "The data is clear. But the part we don't yet know is how much the risk for more aggressive disease is genetically determined and that's the hot area of focus now."

FAST FACTS ABOUT PROSTATE CANCER

- One in six U.S. men will be diagnosed with prostate cancer in their lifetime.
- Prostate cancer is the second most common cause of cancer-related death among men in the United States.
- African-American males experience higher rates of prostate cancer than Caucasian men. Hispanic and Asian men have lower rates of prostate cancer than either black or white men.
- According to the most recent data available, some 215,000 U.S. men are diagnosed with prostate cancer each year.

Source: Center for Disease Control and Prevention

GENETIC GENE FUSIONS

The Hunt

There are two main ways to find genes that may contribute to prostate cancer. One is by doing what are called genome wide association studies (GWAS), where markers across the entire genome of individuals not affected by a disease are compared to those afflicted. These studies are done in large populations and tend to find genetic variants that are more common but have a lower risk of causing disease by themselves. The second way is by analyzing segments of the genome that are thought to be important to the disease being studied and doing minute examinations of those portions in families with a high burden of disease. Linkage studies tend to identify rare genetic alterations that significantly increase risk for disease. It was through linkage analysis that researchers discovered the first prostate cancer specific susceptibility gene candidate—*HOXB13*—early this year.

HOXB13

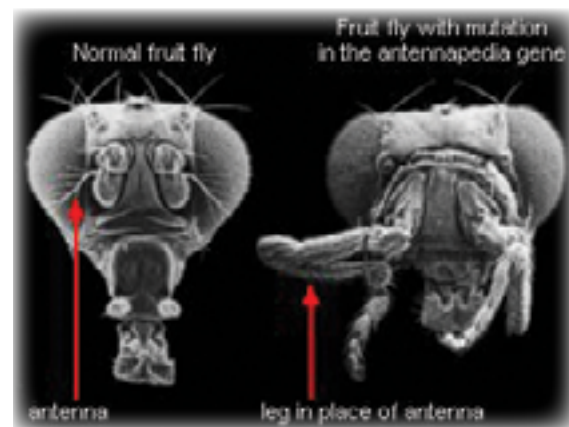
Linkage analyses, including a large study by the ICPCG, kept pointing prostate cancer researchers to a spot on chromosome 17 that was, in effect, “flashing red.” Drilling down, they found what’s known as a missense mutation in the *HOXB13* gene in multiple men in four families that had a history of prostate cancer. In fact, all 18 men with prostate cancer in those four families were carriers of the mutation.

“What’s unique about the *HOXB13* story is that we found a recurrent mutation transmitted over time throughout European populations,” says Dr. Kathleen A. Cooney, who was the senior author on the *HOXB13* findings published in the *New England Journal of Medicine (NEJM)* this past January.

It’s probably no coincidence that a mutated version of this gene has been connected to hereditary prostate cancer. Famously pictured, a fly with mutated *HOX* genes grew legs on its head instead of antennae. Specifically, *HOXB13* fires up just about the time the prostate gland is developing, says Isaacs who was also a co-senior author on the NEJM paper.

In that seminal study, carriers of the mutation had an astonishing 20-fold increased risk of prostate cancer and diagnosis often came at an earlier age. But a later study that confirmed the association of the mutation with prostate cancer “only” found an eight-fold increased risk of disease in carriers. And, Cooney warns that overall increased risk is very different from the absolute likelihood a carrier will develop prostate cancer in their lifetime. For example, approximately 60 percent of female carriers of the *BRCA* mutations will develop breast cancer in their lifetimes. At this time, a corresponding percentage is unknown for men with the *HOXB13* mutation in relation to prostate cancer. “Once we determine lifetime risk, we can begin to get into genetic counseling for carriers and develop personalized medical recommendations or approaches,” says Cooney.

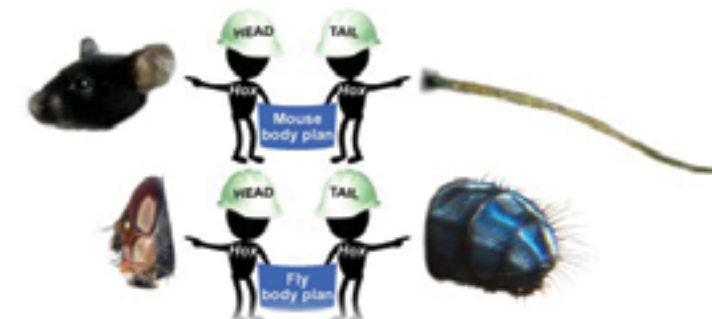
Both Cooney and Isaacs have hefty laundry lists of “To Dos” as they and others study the *HOXB13* mutation. Topping the list is the need to determine how the mutation contributes to the development of prostate cancer. Because the mutation—a change



HOX genes help assure proper assembly of living organisms. (Image courtesy: www.evolution.berkeley.edu)

of only one letter of the gene code that results in a substitution of one protein subunit building block for another—does not cut short the *HOXB13* protein, researchers speculate the mutation may cause an up-regulation of the gene. The resulting “gain of function,” as it is known, would be a classic example of an oncogene where a gene mutates and can churn out excess protein that plays a role in cancer initiation or progression. Researchers will look for increased levels of the genetic products of the gene in the body and discover if the *HOXB13* protein made from the mutated gene hangs around too long in the body before it degrades. A mouse model carrying the mutation will be made and the exact stage the mutated gene kicks into action will be parsed. One theory, says Isaacs, is that the mutant version of *HOXB13*, if inherited, may kick into action at the time progenitor cells for prostate epithelium differentiate and somehow the single change of one letter of genetic code interferes with this in some way, priming the young male for later cancer development.

And while this remains theoretical, if shown to be the case, young men from prostate cancer families could be screened by their pediatrician for the mutant allele, says Dr.



The *HOX* family of genes turns on sequentially during embryo development, determining our up from down and left from right. (Image courtesy: www.evolution.berkeley.edu)

Jonathan Simons, a medical oncologist and specialist in prostate cancer who heads the Prostate Cancer Foundation. And, because the loss of the prostate in mice does not affect fertility, it may be possible to develop the means to ablate the prostate in young men likely destined for prostate cancer—similar to women with *BRCA* mutations who can opt for prophylactic mastectomies.

BRCA

Before the discovery of the mutated *HOXB13* gene, the only major candidate genes for hereditary prostate cancer were the breast cancer susceptibility genes, *BRCA1* and *BRCA2*. These are tumor suppressor genes that when mutated by a truncated protein and subsequent loss of function, can cause breast and ovarian cancer in women and other cancers in both sexes. Researchers studying families with the *BRCA* mutations noticed that prostate cancer cases clustered in male family members and sporadic reports found positive associations between men with *BRCA* mutations and prostate cancer. In addition, prostate cancers in *BRCA* positive men tend to be more aggressive and lethal cancers.

INTERACTIVE RISK ASSESSMENT CALCULATOR FROM THE CLEVELAND CLINIC

The Cleveland Clinic has developed an interactive risk assessment to score your risk of developing prostate cancer. Simply answer the sequential questions as they pop up and an individualized risk score will be calculated that can help you and your doctor decide what steps to take in your screening approach for prostate cancer.

Visit: www.clevelandclinic.org/health/interactive/proassess_risk.asp

Late in 2011 and early in 2012, researchers in the United Kingdom reported more definitive findings on the association of *BRCA* mutations with prostate cancer: men who inherit *BRCA2* mutations have an almost nine-fold increased risk of prostate cancer and 15 percent of all carriers will get the disease by the age of 65; and men who inherit *BRCA1* mutations have a four-fold increased risk of prostate cancer and nine percent of all carriers will get the disease by age 65.

Dr. Rosalind Eeles, from the Institute of Cancer Research and the Royal Marsden in the UK, and an author on both the 2011 and 2012 studies, says that while the latest research shows that in men diagnosed with prostate cancer before the age of 65, only one to two percent will be found to have *BRCA* mutations, it may still be worthwhile to screen for the mutation. A class of drugs called PARP inhibitors has proved effective for the treatment of women with *BRCA*-related breast and ovarian cancers and may be similarly effective in *BRCA* positive prostate cancers, says Eeles. Currently clinical trials in both the US and UK are evaluating how effective PARP inhibitors are against prostate cancer.

Dr. Eeles is also a member of the ICPCG and is heading up the IMPACT study, which is evaluating men with *BRCA* mutations in multiple countries to better understand race prevalence of this mutation and the level of aggressiveness of disease in men with *BRCA* positive prostate cancer. She also says that it is worth revisiting the use of an older chemotherapy drug in *BRCA* positive prostate cancer patients based on new technology-enabled, genetic-based drug screens that show these cancers may respond well to what are known as the platinum chemotherapy agents. "This is a study crying out to be done," says Eeles.

Mismatch Repair Genes (*MMR*)

A distant contender in hereditary prostate cancer genes are a group of four tumor suppression genes known as mismatch repair (*MMR*) genes. Inherited mutations in this group of genes are associated with Lynch syndrome cancers of the colon and other organs. Both Drs. Cooney and Eeles have authored studies analyzing the link between prostate cancer and *MMR* mutations. Eeles and colleagues found that men with *MMR* mutations had a 30 percent risk of developing prostate cancer by age 70, compared to an eight percent risk in the general population. Cooney's study showed a weaker association, but looked only for evidence of disruption in the *MMR* gene in prostate tumors from men with a family history suggestive of Lynch syndrome and not absolute risk. Both Eeles and Cooney say further study it needed to quantify the risk of prostate cancer in men with *MMR* mutations.

But Isaacs points out that, overall, researchers are not seeing a lot of prostate cancer in families that carry *MMR* mutations. Perhaps, he says, because such families are at high risk of being diagnosed with other very aggressive cancers at young ages, prostate cancers tend to remain undiagnosed in this group.

The *MMR* genes act as tumor suppressors by finding and repairing DNA errors of replication. Because it's so important that our bodies be as error-free as possible, evolution has provided us with ecumenical sets of DNA repair pathways in our body. Ultimately, says Isaacs, it may be that only younger men with prostate cancer will be found to have DNA repair problems. "A 55 year-old man who has died of prostate cancer may have fundamentally different problem—it may be that a DNA repair malfunction caused him to succumb to aggressive, rapidly progressing disease—whereas in older men that is unlikely," he says. "Remember, an 80 year-old man with prostate cancer has outlived colon cancer, lung cancer, diabetes, heart disease, etc., so in general, his DNA repair pathways are pretty good."

A SNP Story: Inherited Genetic Variants Specific for Prostate Cancer

HOXB13, *BRCA1*, *BRCA2* and *MMR* mutations linked to prostate cancer have the commonality of being firmly planted within a known gene and are passed down from generation to generation in classic Mendelian fashion in the way of hair or eye color. But smaller changes in DNA, known as single-nucleotide polymorphisms (SNPs), can also be inherited from one's mother and father.

(SNPs are usually identified through GWAS studies that compare a baseline reference genome to a genome(s) of interest—often comparing the genomes of persons with a disease to those without, looking for "variants" in the genome of the former.) SNPs occur most



commonly between genes, rather than in a gene, and can act as potential disease markers on the genetic roadmap; they also can affect when, and if, a gene is turned on or off. A SNP that is inherited is known as a germline (sperm or egg) variant or mutation, because it is present in a person's DNA at birth,

passed down from parent to child. SNPs can also occur spontaneously, as copies of DNA are made throughout a person's life; these are called somatic mutations and are not inherited. Taken alone, a single SNP rarely causes disease in and of itself.

In 2008, a seminal study in the *New England Journal of Medicine*, (senior author, Henrik Gronberg at the Karolinska Institutet in Sweden) found that a panel of five inherited (germline) SNPs were associated with an increased risk for prostate cancer and that risk was dependent upon the dosage of those five SNP variants. That is, a man born with only one SNP had a 50 percent increased likelihood of developing prostate cancer, a man with two SNP variants doubled his risk of getting prostate cancer and a man with four or more of the SNPs more than quadrupled his risk of the cancer. Carrying all five SNPs and a positive family history of prostate cancer increased a man's risk 10-fold.

This Five SNP *NEJM* Study, as it became known, proved nicely that risk for prostate cancer could be genetically quantified, but it had one big problem: not one of the SNPs, either alone in or combination, told researchers if the

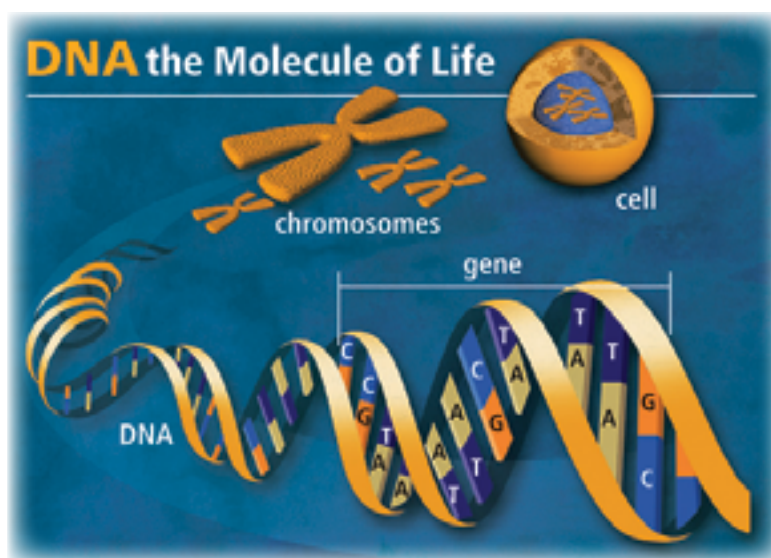
UNDERSTANDING YOUR FAMILY HISTORY OF PROSTATE CANCER

Men are generally considered to be candidates for genetic counseling regarding prostate cancer risk if they have a strong family history of the disease. Prostate cancer can be inherited from a man's mother or father. So in addition to asking about a history of prostate cancer in a man's father and his brothers, it is necessary to ask similar questions about his mother's family. A family history can be as minimal as one member of the family with the disease or many more, with risk of disease increasing as the number of men affected in a family goes up.

If your father or brother has prostate cancer your risk of developing the disease is twice that of the average American man.

prostate tumors likely to develop would be slow-growing non-lethal tumors that would not significantly lower a man's life expectancy, or if the cancers would be fast-growing deadly tumors.

"We took a lot of flack for this study," says Isaacs, an author on the paper. "The research community called us out, saying that it was not really helpful to show increased risk for low-risk tumors as well as high-risk tumors, and we agree," he says referring to the current dilemma of overtreatment of non-lethal prostate cancer. While current diagnostic tests for prostate cancer—such as the prostate specific antigen (PSA) blood test that measures levels of this antigen in men or needle biopsies that examine tissue directly for signs of cancer—are very good at finding prostate cancer early, there is almost no reliable way for doctors to then take that information to the patient and say: well, this tumor is a slow growing cancer and we can just keep an eye on it, whereas this tumor is going to grow like gangbusters and treatment should be fast and furious.



The sequential process known as "The Central Dogma" controls how information stored in our genes is transformed into actual proteins at work in our bodies. (Image courtesy: U.S. Department of Energy)

PNAS) on inherited SNPs, this time looking at some 27,000 SNP variants among 1,980 men with aggressive prostate cancer and comparing them to 2,109 men with indolent, or low-aggression, prostate cancers in what's known as a case to case analysis. For the first time, they were able to show a SNP—rs4054823 located on chromosome 17—that was linked to aggressive prostate cancer.

And while that single SNP only increased the risk of aggressive prostate cancer by 26 percent, it was important as a proof-of-principle study—proving that SNPs that increase the risk of aggressive cancers over non-aggressive cancers actually exist in the genome, says Dr. Karim Kader, a urologist at UC San Diego Moores Cancer Center and one of the study authors. Additionally, the authors found that the risk of aggressive disease increased if the SNP was found on both arms of chromosome 17—remember each of our 46 chromosomes comes in pairs.

But scientists were beginning to understand that one SNP, or even two, does not a prostate cancer make a prostate cancer. The, it-takes-a-panel theme was becoming emergent in prostate cancer genetics and the authors of the *PNAS* paper speculated that men who were found to have a collection of SNPs associated with high-grade aggressive prostate cancer would be good candidates for "targeted" PSA screening, where only men deemed at high-risk for prostate cancer would be tested for prostate specific antigen

Prostate cancer related SNP discovery has currently uncovered more than 40 SNPs associated with prostate cancer risk, but the story those SNPs tell is now only considered truly interesting if they inform on risk of aggressive lethal disease, or to a lesser extent, predict which men with high PSA levels are likely to test positive for cancer on follow up invasive biopsies of their prostates. (Not all men with elevated PSA tests have prostate cancer.)

In 2010 Isaacs, and several of his colleagues from the Five SNP Study, published again (in the *Proceedings of the National Academy of Sciences—*

levels. The idea behind targeted PSA screens is to reduce overdiagnosis of non-lethal prostate cancers. Currently men are not routinely singled out for selective PSA screening based upon genetic testing.

In that vein of thought, a team of international researchers lead by Janet L. Stanford at the Fred Hutchinson Cancer Research Center in Seattle went SNP hunting for inherited genetic variants associated with lethal prostate cancers, searching through 156 genes they felt might hold SNPs with a high risk for lethal cancers of the prostate. After a whole lot of genotyping to look at the sequence of each block of suspect DNA, the team found a panel of five SNPs in five different genes that were strongly associated with deadly forms of the disease. As in other studies, the more the worse, with men who had four or five of the SNPs in the panel at 50 percent higher risk of death from their prostate cancers than men with two SNPs or fewer. This provided further data on the usefulness of groupings of SNPs to provide doctors with indicators of which men might most benefit from PSA screenings based on their inherited risk of developing aggressive or lethal prostate tumors.

Inherited SNPs and Likelihood of a Positive Biopsy

Each year, one million prostate biopsies are done in the US, with less than half positive for prostate cancer. Aside from physical invasiveness of the test, the dollar cost associated with over testing is extreme. Obviously, the ability to increase a physician's arsenal for predicting which men should go on to biopsy and which men can hold off is needed.

Researchers are busy developing many ways to winnow down the population of men who need to undergo biopsy and one hope is that panels of genetic markers will be able to predict with strong accuracy the likelihood of positive biopsy results. This spring, Kader and Isaacs were both authors on a study in the *European Association of Urology* that found some help in using an overall genetic score based upon 33 SNPs that had previously been firmly established as increasing the risk of prostate cancer. (The senior author on the paper was Jianfeng Xu out of Wake Forrest University in North Carolina.) The researchers used a compilation of the SNPs and clinical factors to classify men into three groupings: low risk, intermediate risk and high risk for prostate cancer. When genetic scores were added to clinical findings, such as PSA levels, the ability to predict positive biopsy increased, especially for men of intermediate risk. Alone, the genetic score—calculated by the number of inherited SNPs each man had—surpassed family history at predicting positive biopsy results. The authors note that because a SNP test can be done via non-invasive saliva tests at costs comparable to blood work used to do PSA testing, the modest improvement in risk prediction may be helpful to millions of men. The downside: because the 33 SNPs used in this study are known for their ability to predict the presence of prostate cancer but not the level of aggression, the researchers doubt its value going forward as a predictor of lethal cancers.

UNDERSTANDING YOUR FAMILY HISTORY OF PROSTATE CANCER (CONTINUED)

Hereditary prostate cancer families have even higher risk levels and are defined as having:

A. Three or more first degree relatives have been diagnosed with prostate cancer

or

B. Males from three successive generations of a family have been diagnosed with prostate cancer—e.g., a grandfather, father and brother

or

C. At least two relatives have been diagnosed with prostate cancer at or before the age of 55

Source: Hopkins Criteria, Brady Urological Institute, Johns Hopkins Medical Institutions

SOMATIC GENE FUSIONS

Ancestral Genetic Stories vs. Spontaneous, Ad Hoc Genetic Stories

The genetic story we are born with, passed down from our ancestors, generation to generation is vastly different from the genetic story that develops by chance within a nascent cancer. The above genetic stories of inherited, or germline, prostate cancer genes come to an individual by way of his parents. Alternately, alterations to our DNA that occur after we are born, in cells other than egg or sperm cells, are known as somatic mutations. Somatic means “of the body” and a somatic mutation is one that is not passed down along generational lines. Such a mutation may be harmless, or it may cause cancer or other diseases. Whereas an inherited mutation in our DNA code is found in all the cells that make up our bodies, somatic mutations are only found in specific regions of the body, for example, within a tumor.

And because a tumor is made up of many different regions, just as a country has many states and counties, not all somatic mutations will be found within the confines of the same tumor. Such complexity makes teasing out the genetic mutations that reside in a cancer extremely difficult.

In prostate cancer, like many cancers, certain somatic mutations are found in the majority of tumors and some are very rare. The prevalence of a particular mutation can depend on the race or ethnicity of the person. And “simply” finding a mutation in a tumor does not tell researchers how lethal a tumor is likely to be. Significantly more research must then be done to determine the exact role a mutation plays, if any, in either starting the process of forming a tumor, or in fueling its growth either locally or more menacingly, in distant sites in the body.

TMPRSS2-ERG Fusion Mutations

In 2005, researchers from the University of Michigan and Harvard Medical School discovered that in 23 of the 29 prostate cancer tissue samples they examined, a somatic mutation that fused two genes abnormally with one another occurred. This landmark discovery held up in later work, as independent investigations found this mutation—dubbed *TMPRSS2-ERG*, for the name of the two genes stuck together—in about half of all Caucasian prostate cancer patients. The fusion of these two genes is now considered the most common genetic mutation in human cancers of solid tissue due to the high incidence of prostate cancer.

Because this gene fusion is fairly easy to detect in urine, commercial development of tests such as those used in over-the-counter pregnancy kits is underway. The hope is to add value to the PSA blood test in determining which men are likely to need biopsies when initial indicators show likelihood of prostate cancer. (Remember, elevated PSA levels may or may not mean a cancer is present.) In 2011, the scientists who discovered the fusion gene and their colleagues published a paper in *Science Translational Medicine* showing that urine testing positive for the fusion mutation (and RNA of another gene related to prostate cancer, PCA3) could be used as a means to stratify men as either high-risk for prostate cancers that demand immediate biopsy from men who might best be served by a less aggressive approach that monitors men over time for indicators of progression such as rising PSA levels.

It is still unclear exactly how fusions of the *TMPRSS2* and *ERG* genes promote prostate cancer growth, but researchers have long suspected that the fusion initiates cancer rather than fueling

its growth late in the game. This spring evidence of “how” came to light in a *PNAS* paper. The findings showed that the protein that results from the fusion unleashes a geometric change in DNA’s packaging so that lengthy areas of DNA normally not exposed, and thus not transcribed into proteins, are shape-shifted in a way that aberrantly allows a cascade of genes to be turned “on” and protein products made. It looks to be a case of one +one (*TMPRSS2-ERG* fusion) = hundreds or even thousands of genes abnormally flipped to the “on” position. While hordes of genes switched “on” may seem daunting, the hope is to leverage this discovery into a treatment that stops the initial fusion protein from acting on the packaging of DNA in the first place, keeping the DNA tightly coiled in its normal shape.

As for the enduring question of whether or not prostate cancers that are *TMPRSS2-ERG* positive are associated with more deadly cancers, a large study in *Cancer Epidemiology, Biomarkers & Prevention* this summer found that in men who underwent radical prostatectomy for initial treatment of their cancers, being positive for the gene fusion, was not linked to higher rates of disease recurrence after surgery or aggressive, lethal forms of the disease developing years later. “The presence of this fusion in prostate tissue seems to act as a tipping point toward the development of cells becoming cancerous, but in and of itself, doesn’t weaponize those cells to their deadliest forms that can no longer be contained with local treatments,” says Dr. Simons. “For that to occur, other environmental or genetic events must occur that goad early cancer cells towards aggression.”

SPOP

Another acquired, or somatic, gene mutation unique to prostate cancer cells occurs in the *SPOP* gene and, like the *TMPRSS2-ERG* fusion, is also thought to be an early initiator of prostate cancer. Reporting in *Nature Genetics* this year, investigators from Weill Cornell Medical College in New York and MIT, Harvard and Dana-Farber Cancer Institute in Boston sequenced the genes in 112 prostate tumors and found novel recurring mutations in multiple genes, but mutations in the *SPOP* gene were the most frequent with approximately 15 percent of all tumors testing positive for the mutation.

Unlike genome-wide sequencing (GWAS) that assesses the entirety of the DNA code, the New York/Boston collaboration used a technique called “exome” sequencing that only examines DNA that codes for genes and the proteins those genes make. (Earlier GWAS analyses led the researchers to believe *SPOP* may harbor cancer-causing mutations in prostate cancer.) Normally *SPOP* genes perform housekeeping functions in the cell, such as determining what to keep and what to throw out. The *SPOP* mutations discovered by the researchers occur at places known to be important to this function and may alter how cells tag proteins for disposal. It may be that accumulation of protein trash or failure to retain

WHAT TO DO ONCE YOU KNOW YOUR FAMILY HISTORY OF RISK FOR PROSTATE CANCER

Men from families classified as belonging to a Hereditary Prostate Cancer Family—as defined by the criteria above—should have yearly digital rectal exams and PSA blood tests, beginning at age 40. Men from such families have a 50% risk of developing prostate cancer during their lifetime.

As always, discuss your medical history and family history with your doctor. The choice to screen for prostate cancer should be made by a man in consultation with his doctor.



proteins that check unrestrained cell growth may be early drivers of prostate cancer.

The *SPOP* mutations were found in early stage tumors and did not occur together with the *TMPRSS2-ERG* fusions, thus they are distinct mutations that do not play off one another in forming prostate tumors. Taken together, *SPOP* mutations and *ERG* fusions may account for up to 65 percent of somatic mutation events that drive disease initiation. Whether or not the *SPOP* mutation drives later tumor aggression has not been established.

SLC45A3-ELK4 Fusion RNA

When genes fuse, such as *TMPRSS2-ERG*, it is the offspring protein of that aberrant mating that causes trouble. In the case of *TMPRSS2-ERG*, too much of the *ERG* protein is made which likely leads to cancer initiation in cells. But what about when two distinct RNAs that descend from different genes fuse? Can the resulting fusion RNA cause cancerous trouble? A recent study in *Cancer Discovery* says yes. Looking at prostate cancer cell lines, the study authors found that even though the genes involved (*SLC45A3* and *ELK4*) did not fuse, RNA made from transcriptions of these genes fused together abnormally and the resulting protein-like offspring of this RNA fusion caused cells in culture to grow like mad. And, when they extended their search for this abnormal RNA fusion product to humans with prostate cancer, they found that higher levels correlated with more advanced disease.

SPINK1

About 10 percent of prostate cancer patients make too much of a protein called *SPINK1* in their tumors and this group of men tends to have aggressive forms of the disease. The protein is made from the *SPINK1* gene and under disease-free circumstances should only be expressed, or made, in our gastrointestinal (GI) system organs. Because another GI gene—*HNF4G*—is the master regulator of the *SPINK1* gene and is also actively at work in prostate tumors, the hunt is on for a way to override the *HNF4G* gene in the tumor environment. And because a simple urine test can detect overexpression of *SPINK1*, if a drug can be developed to shut off the *HNF4G* gene, men could be tested for hyperactivity of the gene and shuttled into personalized drug treatments.

SDK1-AMACR et al Fusions as Ethnic Markers for Prostate Cancer in Asian Men

Genetic mutations that drive either the development or progression of prostate cancer vary significantly by ethnicity. For example, while *TMPRSS2-ERG* fusions are found in half of prostate tumors in Caucasian men, only about a third of African-American men carry the mutation, and in Asian men, one-fifth are affected. And a gene that is commonly mutated in many cancers, including prostate—*PTEN*—is mutated less frequently in Asian men with prostate cancer (15 percent) than their Caucasian counterparts (40 percent).

Researchers in China recently discovered a fusion of two genes (*SDK1* and *AMACR*) that seems to be unique to a subset of Chinese prostate cancer patients. This work is early but has important implications for the ethnic diversity of treatments that may come about as mutations specific to race or geography are uncovered. Indeed, this spring, the researcher Dr. Shancheng Ren, who discovered the *SDK1-AMACR* fusion, published along with his colleagues, a study in *Cell Research* that has identified two new gene fusions that are highly endemic in Chinese men with prostate cancer—*CTAGE5-KHDRBS3*, occurring in 37 percent of tumors tested, and *USP9Y-TTTY15*, occurring in 35 percent of tumors.

PCA3 Gene Leads to FDA-Approved Urine Test for Prostate Cancer

This winter, the FDA approved a molecular-based urine test called *PROGENSA PCA3* that aims to advance screening for prostate cancer beyond the limits of PSA testing. The *PCA3* gene was discovered in Dr. Isaacs' lab by Marion Bussemakers in the early 90s, using a then-new technology called "differential display" that compared levels of RNA made by genes. When comparing how much RNA the *PCA3* gene made in normal prostate cells compared to prostate cancer cells, she found that the cancer cells churned out extremely high levels of *PCA3* RNA. It was later discovered that *PCA3* RNA is non-coding, meaning it doesn't produce a protein. "At the time, it was somewhat befuddling because back then we thought most RNAs made proteins," says Isaacs. Yet lack of a *PCA3* protein product didn't undermine the discovery and its use as a prostate cancer biomarker soon became clear: the gene is prostate-specific and levels of the RNA produced by the gene rise dramatically when cancer is present.

A recent study led by the National Cancer Institute showed that the *PROGENSA PCA3* urine test can predict biopsy outcomes in prostate cancer with a high degree of accuracy: a positive *PCA3* urine test predicted a positive biopsy 80 percent of the time at initial biopsy; and, for men undergoing repeat biopsy, a negative urine test predicted a negative biopsy 88 percent of the time.

"The full [commercialization and approval] process took a long time, and was mainly successful due to sustained efforts by Dr. Jack Schalken at University Nijmegen Medical Centre in the Netherlands," says Isaacs, of the new urine test. "It adds to our armamentarium in the fight against prostate cancer, and is an important step in the right direction, but it won't be the end of the story."

The Next Chapters

Almost as quickly as type is set to this page, prostate cancer genes are lining up to tell their tale and finish the story—or at least write the penultimate chapter, because as any good scientist knows, biology often likes a surprise ending. And our genetic stories are unfolding ever sooner. We may not have to wait until we develop cancer to know our proclivity.

This spring, researchers at the University of Washington combined advanced gene sequencing techniques and computational modeling to map the genome of a fetus in utero with just a sample of mom's blood and dad's saliva. (Stanford University followed up with a *Nature* study published a month later, accomplishing the same feat with only mom's blood sample.)

WHAT TO DO ONCE YOU KNOW YOUR FAMILY HISTORY OF RISK FOR PROSTATE CANCER (CONTINUED)

Men with a more limited family history of prostate cancer (such as a father or brother diagnosed with the disease) should consider getting a baseline prostate specific antigen (PSA) blood test* in their early 40s, says Patrick C. Walsh, MD, professor of urology at the Brady Urological Institute at Johns Hopkins and author of *Dr Patrick Walsh's Guide to Surviving Prostate Cancer, Third Edition*, which is published by Grand Central Life & Style (2012). If their baseline PSA reading is greater than 1.0 those men should undergo repeat screening yearly; if their baseline is less than 1.0 at age 40, they should screen again at age 45 and, starting at age 50, screen every year. Men may want to take into account their overall health and life expectancy when making the decision to screen for prostate cancer, says Dr. Walsh. Men with a life expectancy of less than 10 years can generally forgo prostate cancer screens.

*PSA blood tests measure blood levels of a protein produced by cells in the prostate gland. Elevated levels of the PSA protein may indicate the presence of a tumor but remember, not all men with elevated PSA tests have prostate cancer.

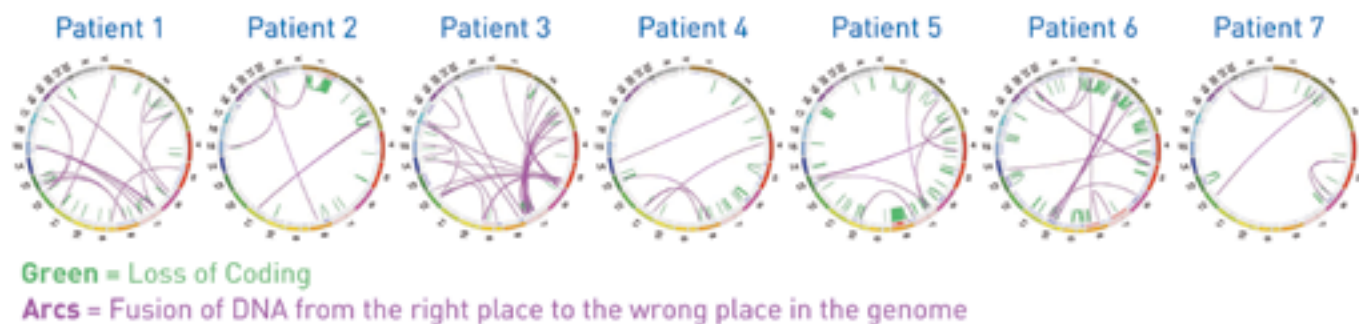
While this may seem scary, and indeed there are potential downsides to knowing too much about our, or our children's genetic futures, the upshots can't be ignored. Waiting until after you've developed cancer to treat it can be too late in some cases. In the case of prostate cancer, says Isaacs, some of the biomarkers associated with aggressive cancer can only be picked up after a man has aggressive disease, at which point any treatment he receives may fail. However, he says, if we had genetic markers that could identify aggressive disease long before it developed, all the better.

And it's not only our genes that affect our destinies, but how we interact with our environment as well. In some cases, what we eat or how much we exercise may cause chemical additions to the outside of our genes that affect their protein or regulatory output. The more we learn about environmental impact on genes associated with prostate cancer, the better able we might be to limit or increase behaviors likely to negate cancer-causing changes to those genes. And perhaps the earlier those changes happen, say, when a child is five or six years old, the less likely he may be to develop the disease as an adult.

As the 21st century moves inexorably onward, our genetic stories may come to us sooner than we'd ever anticipated. But with that knowledge often comes power.



Turning 3 Billion DNA Data Points into an Easy Read

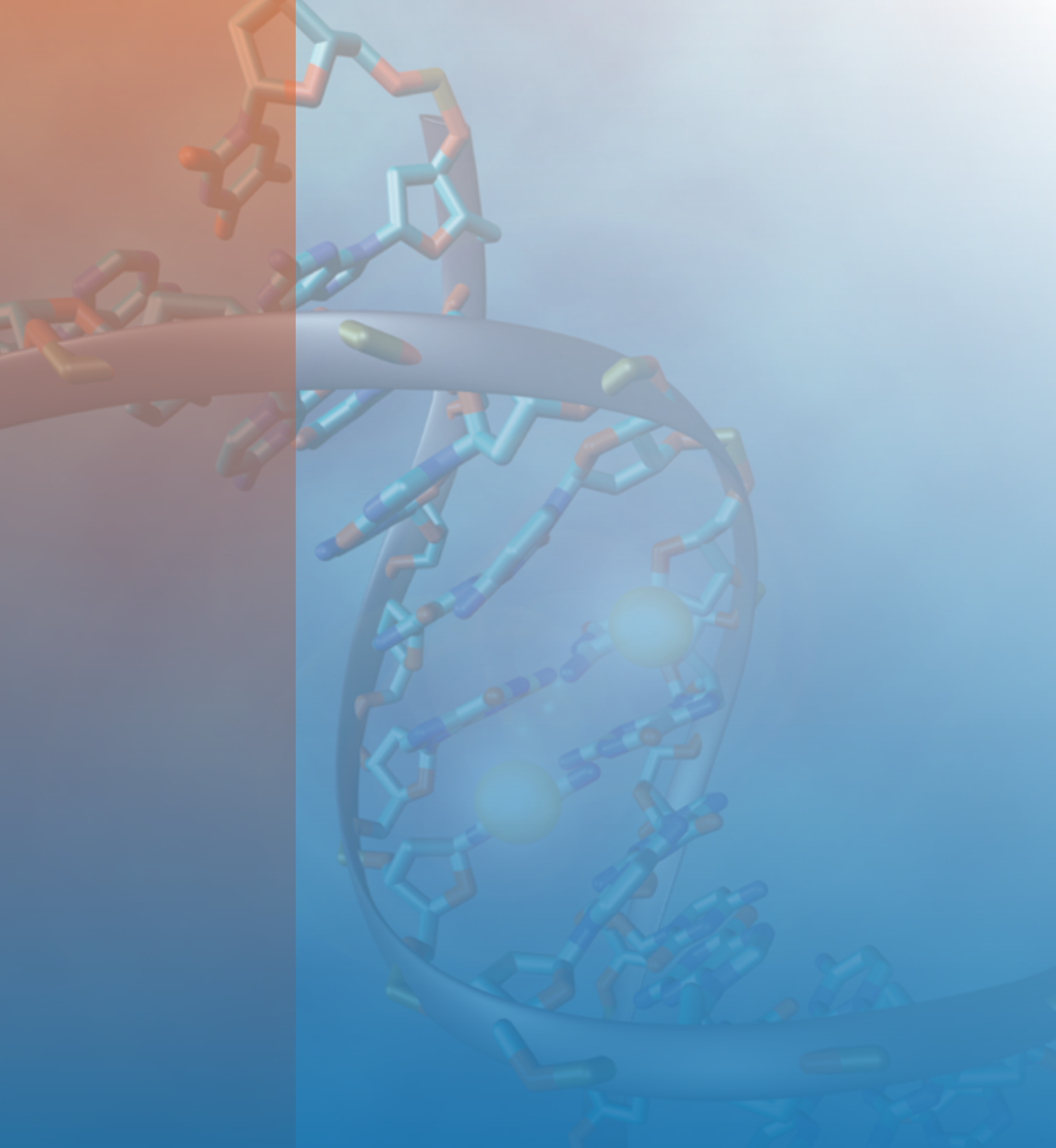


Instead of sifting through a 3-billion letter code, a diagram was developed as a short-hand tool for visualizing important relationships within the genome. It was created with Circos software and is called the Circos plot. Designed with the express purpose of aiding in the visualization of genomic data, its circular layout is analogous to a clock face. Just as a clock face displays time through the use of a fixed dial indicating the hours in a 12-hour cycle, the Circos plot displays the 23 chromosome pairs on which the entire human genome is stored.

These chromosomes are numbered clockwise on the outer circumference of a circle plot. The demarcations within the circle relate information about what might be important for a doctor or patient to know about the genome being studied. On the chart below, the green ticks represent losses of coding information and the purple arcs represent gene fusions, how genetic material goes from the "right place" to the "wrong place" in the genome.

Scientists can quickly assess a Circos plot just as patients can easily glance at a clock face and tell time. Essentially, the Circos plot is an individualized, unique portrait of each patient's prostate cancer, akin to a CT Scan of the entire genome.





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