Dr. Richard Lifton is the Chair of the Genetics Department as well as Sterling Professor of Genetics and Professor of Medicine at the Yale School of Medicine. He founded and is the Executive Director of the Yale Center for Genome Analysis. Prior to starting his laboratory at Yale in 1993, Dr. Lifton received his MD and PhD in Biochemistry from Stanford University and trained at the Brigham and Women’s Hospital in Boston.

Using genetics and genomics, Dr. Lifton’s research has helped identify key genetic factors and biochemical pathways involved in cardiovascular and renal diseases. His findings include the identification of the mechanism linking high salt intake to renal failure and hypertension.

In this interview, Dr. Lifton discusses the importance of the rising field of personalized medicine and how individual- and population-based approaches can shed light on the genetic and environmental factors contributing to the etiology of human disease.

Dr. Lifton, your lab works on uncovering the genetic factors that contribute to cardiovascular and renal diseases. Could you tell me a little bit more about your lab’s interests and some of its current projects?

In the broad context of thinking about personalized or, perhaps a more popular term that’s emerging, “precision” medicine is the notion that the more we understand the specific causes of diseases in individual patients, the better we will be able to devise approaches to diagnosis, treatment, and prevention in those individuals. To the extent that genes are contributing to disease pathogenesis, we ought to be able to figure out what those genetic contributions are, with the expectation that in some cases the genes themselves might be targets for therapeutic or preventive intervention. Moreover, we recognize that genetics is not the sole factor that contributes to pathogenesis. There are undoubtedly environmental factors that contribute as well. But we suspect that at least in some cases, knowing the genes that are driving pathogenesis will help us identify environmental factors that those genes are interacting with.

We now recognize that there are about 21,000 protein-coding genes in the human genome and that a complement of 20,000 or so genes is almost the same set in all vertebrates. It’s not the case that only 10 percent of our genes are shared with everyone else. We have almost exactly the same set. The immune system is one of the more rapidly evolving sets, which makes it a little bit of an outlier. Of course, this is driven by infectious agents that the immune system is responding to. But in general, all vertebrate species are dealing with the same parts list. Given that these genes have been conserved for 4 million years of evolution, it seems rather obvious that there are going to be phenotypic consequences from the mutation of virtually all of these genes. If you ask “where are we today in that quest?”, we know the consequence of mutations in about 3,000 of those genes. So when asked, “What remains to be done in human genetics?”, the answer is, “Practically everything!” We know almost nothing. We know only the more obvious genes that are contributing to disease because they cause very large effects on phenotypes with very high penetrance — and those are the classic Mendelian traits.

In thinking about the kinds of projects that we have been involved in, we started very early on with a focus on hypertension because it is a disease that affects 1 billion people worldwide. It contributes to 17 million deaths per year from heart attacks, stroke, and congestive heart fail-
ure. Cardiovascular disease remains the leading cause of death in the United States and worldwide. We thought hypertension was particularly interesting to study from a genetic standpoint because people couldn’t even decide what organ systems are driving elevated blood pressure. So we looked at the most extreme outliers for the highest and lowest blood pressures compatible with survival and have used increasingly sophisticated technology to identify genes that are driving blood pressure to the high and low end. We have shown that, in fact, these genes converge on how the kidney handles salt. Genes that increase salt reabsorption by the kidney raise blood pressure, and genes that reduce salt reabsorption by the kidney reduce blood pressure. There are diverse effects on potassium, calcium, and magnesium homeostasis, but if you know what’s happening to sodium and chloride reabsorption, you know what’s happening to blood pressure. This has had an impact on how we think about prevention in the population because it immediately identified an environmental covariate: dietary salt. Also, in the case of these rare patients with specific genes that are driving their blood pressure, it suggests very specific approaches to their individual treatments. But it also has identified new targets and combinations of therapies that are now widely used in the general population, as is the prevention strategy. There are 30 countries that have now adopted approaches to dietary restriction of sodium chloride to try to prevent the development of hypertension and prevent morbidity and mortality from cardiovascular disease. That’s a project that’s been running in the lab for 20 years now.

In 2008, as the technology improved, and with a company called NimbleGen, we began developing techniques for selectively capturing and sequencing just the protein-coding parts of the human genome. While genome sequencing was still very expensive, we thought that there is a lot you can do if you just look for mutations in protein-coding parts of the genome and start to flesh out the human genetic map to identify the links between mutations in specific genes and diseases. So we thought very specifically that if there is a phenotype resulting from mutations of virtually any ostensible gene, why haven’t we previously recognized these as single Mendelian traits segregating in families? It occurred to us that one obvious place we ought to look is in dominant reproductive lethals, meaning that if you get a single mutant copy of the gene, you are very unlikely to ever pass that mutation onto a next generation. This is either because of lethality in the embryo or in the individual before reproductive age. Typically, those would be de novo mutations that would arise from mutations absent in the parent and present in the offspring, and we’d have no ability to find those by traditional mapping approaches. We thought de novo mutations causing reproductive lethality was one place that we would systematically not have been able to look.

A second place would be diseases with incomplete or low penetrance. Not everyone who gets the mutation gets the disease. As a consequence, you might not recognize that these are Mendelian traits in families. Good examples of those would be diseases in which you’re just fine with this mutation unless you get a particular environmental provocation such as an infectious agent, smoking, or exposure to dusts and molds of various kinds.

And third, we thought there are likely many rare recessive diseases that are sufficiently rare because of lethality that typically we haven’t recognized Mendelian patterns of segregation. As a consequence, we’re very interested in looking for genes in patients that have extreme phenotypes that arise in the setting of consanguineous union. In the last 5 years, we’ve been exploring these different areas. It turns out that these are incredibly fruitful for the identification of new genes that are causing human disease in a wide range of areas. We’re one of three centers funded by the NIH (National Institutes of Health) to develop new strategies for finding rare mutations with large effects. Over the last 3½ years, we have sequenced about 10,000 samples as part of this national effort. Our group has identified about 150 new disease traits in different areas. Probably among the most interesting of those would be congenital heart disease and work by Matt State, when he was here at Yale and continuing now that he’s at UCSF, in autism. These are probably the two best-studied traits by this strategy so far. Starting with healthy parents and a single severely affected offspring and asking, “Do you find an excess of de novo mutations in patients with these diseases in particular genes or pathways?” In the case of both autism and congenital heart disease, the answer is that about 10 percent of patients have their disease due to a single mutation.

Most interestingly, the pathways that are implicated in these two diseases are the same: mutations in chromatin-modifying genes. We know that key genes involved in developmental pathways are regulated by turning enhancers and promoters on or off by specific covalent modifications of histone proteins in chromatin. It turns out that these are commonly very dosage-sensitive genes so that haploid insufficiency — loss of one copy of the gene — is sufficient to have a large phenotypic effect on a system, like the development of the heart or the development of the brain. This has been an area of great interest over the last couple of years.

But we’ve also been applying this to ostensibly everything that we think is tractable and a good idea. A major area of interest, as a consequence, has been neoplastic diseases of diverse types, ranging from cancer to benign tumors that also make a hormone. In the case of hypertension, there are about 10 million patients worldwide who have tumors of the adrenal gland that constitutively make the hormone aldosterone, which is a hormone that tells the kidney to hang on to salt and water. It causes hypertension. We always were interested in the possibility that there might be simple mechanisms that link the cell proliferation and the cell-autonomous production of the hormone. We tested this, starting with the assumption that these tumors are clonal, which is a fairly standard assumption in neoplasia, and that if that’s true, there ought to be somatic mutations present in the tumor that are not present in the normal cell. That turned out to be true. We’ve now shown for three hormone-producing tumors (aldosterone-producing tumors of the adrenal gland, cortisol-
producing hormones of the adrenal gland, and insulin-producing tumors of the pancreatic beta cells) that single mutations are sufficient to drive the pathogenesis of these tumors. There is a deep link between the signals that tell these tissues to make their cognate hormones but also tell them to proliferate. It makes teleological sense that if there are ongoing metabolic demands for the hormone, the body would want to support that by making more of the cell types that can handle the load. This is the case for each of these tumors.

In the case of tumors that cause hypertension, there are two mutations in the same gene that commonly are found and that account for nearly half of these tumors worldwide. Quite an unexpected finding. These are mutations in the potassium channel that cause the channel to be permeable to sodium on top of its normal function in transporting potassium. This leads to cell depolarization, which is the signal that activates the voltage-gated calcium channel. Increased intracellular calcium is acutely the sufficient signal to make aldosterone and chronically to cause cell proliferation. That’s what happens in these tumors. They are a consequence of the constitutive activation of these voltage-gated calcium channels. One of the personalized medicine aspects of this is that we think that there’s great potential to be able to diagnose these tumors by finding these specific mutations that are only found in these tumors, in cell-free DNA circulating in the plasma of patients. If we can do that, we could make the diagnosis of these tumors vastly easier. Right now, in most patients with these tumors, to make a diagnosis that would enable you to take the patient to surgery, you need to find a tumor in the adrenal gland and show by adrenal vein catheterization — by threading a catheter up into the adrenal veins — that there is aldosterone coming in one side and not the other, an invasive procedure only done in a handful of places. Most patients with these tumors consequently don’t get diagnosed until very late in their course, if at all. If you could diagnose them by a simple blood test, that would be a huge advance.

On the rest of cancer, we’re, as a field, deep into the identification of the mutations that are driving each specific cancer type. It turns out there is tremendous diversity in the genes that are implicated. There are around 330 different genes that when mutated contribute to cancer of different varieties. Some are very frequently mutated, like p53 and Ras, and there are others that are very cell-type specific and only play a role in tumors of that cell type. This is profoundly changing the way in which we’re thinking about both classification and treatment of tumors. We expect that we will be able to develop predictive biomarkers of who’s going to respond to which treatment when we have enough tumors that are sequenced and understand their clinical outcomes. This is one of the major goals of the precision medicine initiative that President Obama announced in the State of the Union address this year. I think there’s enormous opportunity for continuing to try to link genotype to phenotype using intermediate metabolic markers or cell type markers from the immune system in this process, but we’re incredibly early in this.

The other area that we expect this kind of personalized or precision medicine to make contributions is in response to therapy. We know there’s a wide variation in the response to treatments of all sorts. We also know that the adverse effects that result from the use of drugs is highly variable among patients. We already have some very good examples of severe adverse effects that result if you have a drug and you have a particular mutation that interferes with the metabolism of the drug so that you get very toxic levels or idiosyncratic reactions. We expect to be able to flesh that out with the study of large numbers of individuals who are going onto various therapies. In the precision medicine initiative, we expect to study at least a million people and have complete longitudinal health care data as an ongoing longitudinal study coupled with genome sequence data, metabolic markers, and increasingly sophisticated data about the immune system, for example. With this large database, we expect to be able to make novel linkages between genotypes, intermediate biomarkers, phenotypes, and ultimately disease outcomes.

These are exciting and realistic prospects limited only by cost at this point. Very significant financial resources will be needed to complete these studies. But the cost of doing them will be drastically lower now than they were 5 years ago because of the wide use of electronic medical records and the very low cost of genome sequencing. The time is right to be making a very big push on this. This is in the context of the same historical sweep that we’ve been in, in the last several thousand years. Our ancestors long ago thought that all diseases were caused by one unifying evil humor in all its manifestations. We’ve been dividing up diseases into smaller and smaller slices based on distinctive pathogenesis. I think we now have the tools to get down to the very specific causes of disease in individual patients, with the expectation that in many of those patients, this is going to provide definitive treatment for the disease or preventive approaches that will keep them from getting the disease at all.

That sounds very exciting. As you mentioned, the cost of sequencing has plummeted since the Human Genome Project — and we know how much that cost — compared to what it can cost nowadays.

Just to put a fine point on that: When we started sequencing the human genome in 1998, it cost $100,000 to sequence a million bases of DNA. This year, it cost about a penny to sequence the same number of bases. So seven orders of magnitude in reduction in cost. This outstrips Moore’s Law in computation.

This plummeting cost has allowed us to better identify genes that can factor into disease development. Finding the right cohort can be quite limiting, for instance. What other important hurdles might there be, in your opinion?

I think having good phenotypic data coupled to genotypic data and having that over time is really important. But your starting point is where we’ve been with the Mendelian project. Over the last 3½ years, any time we saw a patient
who has a disease that no one has seen before or doesn’t quite fit into any known syndromic pattern, we sequenced them, not with the expectation that we’re going to figure it all out, but that we’re going to figure out some of the cases.

There’s a very interesting example that we published last year that came from Yale-New Haven Hospital. There was a 15-day-old boy referred to Yale with a high fever and watery diarrhea. He was recognized to be very sick and getting sicker, and no one knew what the diagnosis was. Infectious causes had been ruled out. He was developing coagulopathy and anemia. Obviously, his doctors were very concerned that he was not going to survive. After being contacted by his doctors in the intensive care unit, we sequenced the parents and the offspring and we didn’t find any de novo mutations of note in the patient, but we did find a previously unidentified mutation in the gene \textit{NLRC4}. This is a gene in the inflammasome, one of the core components of the inflammatory response. That tied to the clinical recognition by one of the attending physicians, who thought the patient had signs of systemic inflammation. Measurement of interleukin-1 (IL-1) and interleukin-18 (IL-18) levels turned out to be very high. Other inflammatory markers were very elevated as well.

Several days after the baby’s funeral, the father ended up intubated in the intensive care unit at an outside hospital with a high fever, coagulopathy, and no obvious source of infection. He shared the mutation with his son, in \textit{NLRC4}. The mutation in him turned out to be de novo. It was absent in his biological parents. The father ultimately was put on high dose immunosuppression and recovered, at which point his medical history was probed further. And he said, “Well, yes, in fact, I’d been hospitalized at Boston’s Children Hospital at birth with high fever and a GI problem that was never well explained.” He’d been written up at birth as an unusual case of watery diarrhea with high fever. Every time he was put on steroids, he would get better. When the steroids were removed, he’d relapse. He was one of the first children in the United States who was given total parenteral nutrition, in which all of his calories were administered intravenously. He had gotten better over the years with the exception that he still got a very high fever when he incurred physical or emotional stress. In retrospect, the death of his son had clearly triggered this auto-inflammatory syndrome. He has a son by a different mother who also has severe periodic fever syndrome that was unexplained.

This is an example of where the clinical recognition of an unusual patient led to the description of the disease, the identification of the gene that caused the disease — there are now two other families that have either the identical mutation or a different activating mutation in \textit{NLRC4} — and a treatment. There’s an existing therapy that uses monoclonal antibodies to IL-1 that reduces its levels and prevents the chronic inflammation going on in these patients. That’s a nice example of where the technology comes together and suggests a therapy already on the market. I think there will undoubtedly be many more examples like this as we go forward. The father and the son were not even recognized to have related clinical problems beforehand. So you can see the difficulty in trying to recognize that as a distinct Mendelian trait until you have the answer.

\textbf{It’s a very touching example of how precision medicine can lead to these types of discoveries. How often do you see your findings or these types of findings from precision medicine actually lead to therapeutic advances or preventive advances in the field?}

Certainly in the case of hypertension, the work has led to preventive efforts worldwide based on the knowledge of the relationship between salt and blood pressure. I mentioned congenital heart disease and autism. The finding that the same pathways of histone modification are mutated in both congenital heart disease and autism suggests there may be an underlying link that has not been well recognized between these two diseases. In fact, it’s known that after surgical correction of severe congenital heart disease, almost half of these patients will have neuro-developmental abnormalities. So these findings raised the possibility that the underlying biology, namely which genes are mutated, may be the determinant of which patients are going to be prone to neuro-developmental abnormalities with congenital heart disease. If that turns out to be true, one will be able to identify these patients early on and hope to intervene in ways that would prevent or mitigate the neuro-cognitive and neuro-developmental abnormalities. I think that this is an interesting possibility going forward.

Obviously in the case of cancer, immunotherapy is clearly having an enormous impact on how we’re thinking about treating solid tumors. It first raises the question of what is the profile that predicts who is going to respond to therapy and who is not. Second, can we do a better job of predicting not only who is going to respond but also who after responding is likely to relapse or what the causes of relapse will be? Are there mutations that arise that now make the tumor resistant to immunotherapy and, if so, can we identify ways to mitigate that? In immunotherapy, only a small fraction of patients respond. Is that going to be a concrete wall that we won’t be able to penetrate or will we be able to find combinations of other factors, in addition to immunotherapy, that will turn response rates from 20 percent to 70 percent or eventually approaching being able to treat everyone with immunotherapy as a cornerstone of treatment? I think there are enormous possibilities there.

\textbf{It sounds like it. Your work focuses more on the genetics that affect disease development, although you mentioned that this all happens in a given environment. Given that, what do you think about the growing research, within precision medicine, on the microbiome as contributing to human disease? A lot of it is correlative. There is very little causation found. Nevertheless it’s still such a hot field at the moment.}

I think the animal work is quite convincing in that in certain contexts there are causal relationships between the
microbiome and traits in animals. How that will translate to humans is presently an open question, in my opinion. There are tantalizing lines of evidence that clearly indicate that in some contexts the microbiome is critical in disease pathogenesis. But how far that will extend is unknown. The challenge will be, exactly as you said, going from correlation to being able to establish causation. But infectious agents obviously give the ability to manipulate the microbiome and determine whether you can mitigate or alter the biology by changing it. These are doable experiments, and we just need to get on with it and do them.

**I don’t know if this is actually implemented systematically in hospitals, but there are thoughts about using fecal transplants for really extreme cases.**

*Clostridium difficile* is a severe infectious agent in the intestine. This is a case in which fecal transplants are being applied, but it may end up that you can just prevent *C. difficile* infection by using particular bacterial strains capable of recolonizing the niche or maintaining the niche by antibiotic treatment. I am very enthusiastic about those specific kinds of examples.

**Is there anything you think is important for our readers to know?**

As I think about the future of therapeutics, there are two general models. One in which specific knowledge of the individual’s genotype is critical and one in which it may not matter at all. I’ll frame these as “the cancer model” and the other model as “the cardiovascular model.” In the cancer model, because single mutations with large effects are frequently drivers of cancer, hitting single gene targets is very suited for therapeutics. We now have a good example of the recurring *abl* mutations in chronic myelogenous leukemia, recurrent epidermal growth factor mutations in lung cancer, the recurrent *alk* mutations in lung cancer, and *BRAF* mutations in melanomas, as examples in which, if you know the genotype of the patient, there are specific drugs that can be given to inhibit the activated gene product. That has a large impact on response to therapy. In each of these cases, we have specific drugs that target the mutated gene product that has the mutation but also typically the wildtype gene product. Regardless, you find the mutation and give the drug. The drug doesn’t work in other patients. It only works if you have the mutation. That’s the cancer model. Personalized or precision medicine is really clearly important there.

The other model is one in which you have common diseases that may not frequently have single mutations with large effect. But if you find the major pathways that can be manipulated and have large effects with few adverse consequences, you may be able to treat millions of people regardless of what their underlying genetic basis is. Good examples of that would be LDL cholesterol and risk of heart attack and blood pressure and risk of heart attack and stroke, where we treat millions of individuals with drugs that lower LDL cholesterol and blood pressure. The knowledge that we’re hitting the right targets has come from rare mutations in patients that have very large effects on these traits. In LDL cholesterol, the new kid on the block is the recognition of mutations in the gene called *pcsk9*. Gain of function mutations in *pcsk9* causes elevated LDL cholesterol, and loss of function mutations in *pcsk9* reduce cholesterol. Based on this, along with the knowledge that if you’re missing *pcsk9*, you’re apparently healthy and normal with the exception that your LDL cholesterol is low, monoclonal antibodies to PCSK9 that lower levels of the LDL receptor were developed. This turns out to be a terrific injectable drug to lower LDL cholesterol. Everybody expects that this will have very large effects to reduce risk of heart attack when given alone or in conjunction with the inhibitors of the rate-limiting enzyme for cholesterol biosynthesis, HMG-CoA reductase. Those would be examples in which you find, via extreme outlier genetics, the genes and pathways that can be manipulated and can be applied to millions of individuals regardless of whether they have mutations in this gene.