Welcome to the 2012 UCSF Lupus and RA genetics research projects electronic newsletter!

As you know, research like this would not be possible without the help of individuals like you - thank you! We are turning over a new leaf (so to speak) and distributing newsletters completely electronically! Not only will this save trees, but it will also allow us to send out updates to all of you on a more regular basis.

Several new advances in genetics research have revolutionized the way we look at genes. In this newsletter, you will read about the numerous ways we have adapted to these advances from recruitment to analysis, as well as in our study design.

We are fortunate to work closely with several well-respected researchers at UCSF and at UC Berkeley. Here, you can read about what goes on in the genetics lab itself and learn about new research from some experts in the field of molecular biology. You can also learn about some very important findings related to kidney disease in Lupus and about a new RA study - soon to be underway!

We hope you enjoy reading this update!

New Chief of Rheumatology Division!

We are pleased to announce that Dr. Lindsey Criswell has accepted the position of Chief of the Division of Rheumatology at the Parnassus Heights campus and assumed this role on July 1, 2011. Dr. Criswell received her M.D. from UCSF in 1986 and an M.P.H from the University of California, Berkeley in 1992. She completed a post-doctoral fellowship in Rheumatology at UCSF and joined the Rheumatology faculty in 1992. Dr. Criswell currently holds the Kenneth H. Fye Endowed Chair in Rheumatology in recognition of her research and mentoring contributions.

Dr. Criswell is an internationally-recognized leader in the search for genetic and environmental factors that contribute to the development and progression of autoimmune diseases. Dr. Criswell is helping to lead an international effort that involves genome-wide scans to identify novel genes that increase the risk of lupus and rheumatoid arthritis. These studies involve hundreds of thousands, and sometimes over a million genetic markers and are among the first such projects worldwide for any disease. She and her collaborators have already identified more than 30 genes that appear to be important in predisposing people to rheumatoid arthritis and/or lupus. She is currently extending this work to another autoimmune disease, Sjögren’s Syndrome, which has been relatively understudied.
A New Field of Study: Epigenetics in Autoimmune Diseases  
by Sharon Chung, MD MAS

Recent breakthroughs in the field of genetics have led to astounding advances in understanding how genetics contributes to the risk of developing lupus and rheumatoid arthritis (RA). Currently, over 30 genes have been identified as influencing a person’s risk for developing lupus, and over 25 genes have been identified for RA. With your help, our research group has participated in many of the studies that have identified these genes. However, even with these successes, the entire genetic risk of lupus or RA cannot be explained. Therefore, our group is now investigating how other types of genetic variation contribute to the risk of developing these diseases.

Epigenetics is a new field of genetics research increasingly being investigated to understand factors that influence the risk of developing autoimmune diseases. Epigenetics refers to methods of changing the expression of a gene without having to change the DNA sequence of that gene. One particular epigenetic method being studied by our group is DNA methylation. In DNA methylation, methyl (carbon) groups bind to certain DNA sequences and influence gene expression. When the DNA for a gene is not methylated, the gene can be expressed. When the DNA for a gene is methylated (that is, the methyl group is bound to the DNA), gene expression can decrease and the gene can even be silenced.

DNA methylation patterns are determined in the developing fetus. However, these patterns can change over time. Methylation patterns change as individuals age and differ between men and women. In addition, methylation patterns are influenced by diet and environmental exposures such as smoking and sun exposure. DNA methylation is especially intriguing in lupus, since drugs that can cause lupus in susceptible individuals are known to change DNA methylation patterns. In previous studies of DNA methylation in both lupus and RA, inflammatory cells from patients with active disease have been shown to have less methylation than cells from healthy individuals, suggesting that genes are “turned on” too much in patients with these autoimmune diseases. Most importantly, in other diseases such as cancer, drugs that are known to change DNA methylation patterns have been successfully used to control disease. Therefore, understanding how changes in DNA methylation affect the development of lupus and RA may lead to new targeted therapies for these diseases.

In our group, we have started two studies in DNA methylation. The first study investigates whether changes in DNA methylation are related to whether or not an individual with lupus produces a particular antibody (anti-dsDNA autoantibody) which is associated with kidney disease and more severe lupus. Our study thus far has shown that changes in the DNA methylation of three particular genes are associated with the production of this autoantibody. What is particularly exciting is that these three genes have not been identified as risk genes in other genetic studies of lupus. Therefore, our studies of DNA methylation are providing new insights as to why lupus develops. Most recently, we have started new DNA methylation studies in Sjögren’s Syndrome as well as in RA. These studies, supported by the Sjögren’s Syndrome Foundation and the American College of Rheumatology Research and Education Foundation, will investigate how DNA methylation influences the risk of developing Sjögren’s Syndrome or RA, as well as look at factors that influence disease severity.

Our hope is that these new studies will improve our understanding of how lupus, RA, and Sjögren’s Syndrome develop, and identify factors that can be targeted in the next generation of medications for these diseases. These studies, like the others described in this newsletter, would not be possible without the contribution and support of the participants in our genetic studies of autoimmune disease - thank you!
An Interview with Averil Ma and Barbara Malynn
by Kirsten Sterba

I had the privilege of sitting down with Dr. Averil Ma, the Rainin Distinguished Professor of Medicine and the Chief of Gastroenterology at UCSF, along with his wife Dr. Barbara Malynn, a specialist in Gastroenterology at UCSF, to discuss their research about the role that the A20 protein pathway plays in autoimmune disease. They are experts in the field of molecular biology, with 20+ years of experience each and we are lucky to collaborate with them to better understand SLE and RA. When they are not busy in the lab, they enjoy hiking at the Pt. Reyes National Seashore.

Q1: What sparked your early interest in science?

Dr. Ma: I have always been interested in the mechanisms of disease, why diseases happen, and understanding them at a molecular level. It has been an interest and long effort of ours to try to understand the molecular mechanisms of disease, which includes the genes that encode the proteins that cause the disease as well as how the proteins work together in cells and how those cells then behave badly to cause disease.

Dr. Malynn: I would have to agree with that and I would add that simplistically, when in college and deciding where to go, I always wondered why is it that some people get sick and other people don’t. At the time that we were coming up [in the field of molecular biology] that basic question was still unclear because we were just starting to learn about molecular biology and the genetics of it was still in its infancy.

Q2: Can you describe your research to our RA and SLE participants? What do you think would be of greatest interest to them?

Dr. Ma: The A20 gene is one of the very few genes that is strongly associated with susceptibility to developing both RA and SLE. Even though they are very different diseases this gene is particularly important to both and we do not know yet whether it is through a common mechanism or through multiple mechanisms. It is important to know how this molecule functions genetically to cause disease. Secondarily, RA and SLE patients may be interested to know that both genetic changes that you are born with and genetic changes to the gene that happen afterwards in the course of cells dividing also cause lymphomas, cancers of the B-cells [which are an important part of the immune system], which is one of the most serious complications that RA and SLE patients are at risk of getting. This gene appears to perform many functions that can protect us from disease, but when the gene is not properly functioning it can predispose patients to get the disease along with certain complications. For instance, in SLE, the A20 gene is also linked to getting the serious kidney manifestations.

Dr. Malynn: We are just starting to work with geneticists, like Dr. Criswell, to correlate these genes to disease severity and response to treatment. I think it is also important for patients to know that we can take the data that is generated in Dr. Criswell’s research and test them experimentally in mice to see if these changes in the genes lead to changes in the mice and we can use these mice to test for ways to intervene in the disease. There is uniqueness to the collaboration we have with Dr. Criswell in that we are really able to inform each other.

Q3: Describe your collaboration with Dr. Criswell.

Dr. Ma: There are two very important collaborations with Lindsey. The first is that we took cells from SLE patients who were so generous to volunteer. We can directly test these cells in the laboratory to see how they behave and the abnormalities that may occur in these cells that are caused by genetic changes in the cells. And not only in genes like A20 but also in related proteins like ABIN (binding protein of A20, also associated with SLE). The other direction that we collaborate in is performing more physiological studies in the context of the whole animal by genetically engineering mice to have different mutations in these genes and then create, therefore, in a whole intact mouse, a model of the human condition. These mice can develop different RA and SLE-like conditions and then we can study the pathophysiology of the disease in addition to studying individual cells.

Q4: Can you provide a broad outline of how discoveries with the A20 gene could lead to better therapies for RA or SLE patients?

We have found that either reduced or absent levels of the A20 gene increases susceptibility to getting RA or SLE. It would be nice if we could just give someone a drug that would increase or replace this absence of A20, but we can’t. What we can do is try to figure out ways to develop drugs that will inhibit specific proteins or knock them out, but that takes a lot more time. That is why we have not gone as quickly from these observations to developing a cure. However, even though the path is a little longer we have already found other proteins that inhibit A20’s activity, so if we take the slightly more indirect route of inhibiting the inhibitors of A20 then one might imagine that the net effect would be to raise A20 levels, and therefore we would have a therapy to prevent both RA and SLE from occurring. (cont on page 5)
In 1997, Dr. Criswell began her research and some of our first lupus and RA patients were enrolled in the UCSF Lupus and RA Genetics Projects. In the 15 years since then, genetics research has evolved in ways we couldn’t have imagined in the beginning. The biggest change by far is in the amount of genetic information we are able to generate and analyze.

First, let’s back up a bit to what that genetic information looks like. As you may know, humans have 23 pairs of chromosomes that contain our genes. You can think of each chromosome as a very very very long chain of beads. Each bead is a molecule called a nucleotide, and these nucleotides form genes and give our body the instructions to produce proteins, which affect everything from our eye color to how we react to foreign substances that enter our body. We each have billions of these “beads”, in every cell of our body. If you look at any two people, it turns out that more than 99.9% of these beads contain the same information, i.e. are the same nucleotide (there are four - adenine, thymine, cytosine, and guanine). It is the ones that vary among people – the less than 0.1% that are different - that we study. We look for which of these genetic variations are associated with getting lupus or RA, or which clinical attributes are present in different lupus or RA patients, or which lupus or RA patients will respond well to a new medication.

Back to the changes that have happened in the past 15 years. There have been truly dramatic advances in the laboratory technologies used to determine our genotypes – i.e. which bead or nucleotide is present for a particular gene variant. These improvements in technology make it much much faster and much much cheaper to determine each genotype. In 1997, a genetics research study might have studied 3 to 10 genetic variants, located in a few genes, in a few hundred people. Five years later, perhaps we would study 100 variants at a time. By 2009, for similar cost, we would study ½ a million genetic variants spread across the entire genome (a “Genome-Wide Association Study”, or GWAS), located in many thousands of genes, in a few thousand people. Today’s GWAS technologies typically cover 2.5 million variants. And the latest technology, “Whole-Genome Sequencing” (WGS), has the capability of determining all of the gene variants within each person. The cost of WGS is plummeting, just as researchers are beginning to generate and study this type of data.

Then, of course, we must analyze the massive amount of data generated from GWAS and WGS studies in order to answer our research questions. So along with the evolution of genotyping lab technologies, there has also been a rapid evolution of statistical methods and computer software – which we are constantly challenged to keep up with. A very important aspect of all of this data is that the greater number of participants we have in our studies, the more accurately we can interpret our results. The larger our study population is, the easier it is for us to “separate the signal from the noise”. So our research participants are more important than ever in this new era!

New NIH Funding for The LOS Study!

Many of you who have participated in the SLE Genetics Study have gone on to become part of the Lupus Outcomes Study (LOS). Our purpose was to follow people with lupus over the course of many years, through annual telephone surveys. Over 1,200 people with SLE like you have participated in the LOS and, over the ten years of the LOS, we have completed more than 8,000 interviews.

Through your participation, we have made some important discoveries about the effect of lupus treatments, health care access, and what can improve outcomes. With our new NIH Grant, we’ll be focusing on how genetic make-up affects long-term outcomes of SLE and on how communication with the physician affects the kinds of health care for SLE.

In the next few months, those of you who have participated in the SLE Genetics Study but who have not been enrolled in the LOS will be asked to join in that important study, too. We hope you’ll agree to join the 1,200 other people from the SLE Genetics Study who participate in the LOS when our interviewers, Janet, Stephen, and Jessica contact you!
Kidney Disease, More Common in Some Lupus Patients by Ilana Richman, MD

Among people with systemic lupus erythematosus (SLE) in the United States, about 30% have kidney disease related to SLE, or lupus nephritis. Although many patients who have SLE will never develop lupus nephritis, those who do may have significantly impaired kidney function and may require dialysis or a kidney transplant. Understanding the risk factors for lupus nephritis may help doctors identify patients who may develop this complication and treat them appropriately.

Beginning as early as the 1960s, doctors recognized that minority populations with SLE are at greater risk of developing lupus nephritis. More recent work, including our own studies, have shown that Asian, African American, and Hispanic individuals are at least 50% more likely to develop lupus nephritis than Caucasians with SLE. Although these disparities are well described, the causes are less clear. Both environment and genetics could play a role, though disentangling genes and environment has often proven difficult.

A recent study by our research group aimed to address this question: Why are different ethnic groups at varying risk of developing kidney disease in SLE? Are genetics responsible? Or environment? If genes are at the root, can we identify which specific genes are involved? To answer these questions, we examined the genetic ancestry of a group of SLE patients in relation to the risk of lupus nephritis. Genetic ancestry refers to the geographic populations from which an individual is descended. Using genetic and mathematical tools, we can describe the populations that have contributed to a single individual’s genome. We can then ask whether membership in an ancestral population puts an individual at risk for lupus nephritis.

Our study examined about 1800 people with SLE. We characterized the genetic ancestry for each participant and found that most people were a mixture of ancestries. Most had some European ancestry, some had African, East Asian, or Amerindian ancestries. We observed that the more European ancestry an individual had, the less likely he or she was to develop lupus nephritis. This relationship held even when we accounted for differences in socioeconomic status (such as education level), age, gender, or other factors that might contribute to the development of lupus nephritis. Although we examined a number of genes associated with kidney disease in SLE, we did not find specific genes that explained the relationship between European ancestry and lupus nephritis.

We believe that our results conclusively demonstrate that there is a genetic basis for disparities in rates of lupus nephritis among ethnic groups. We hope that this study will help rheumatologists identify patients most at risk for developing lupus nephritis. Ultimately, we hope to be able to identify the specific genes responsible for lupus nephritis and to develop targeted therapies to address this serious complication.

(Dr. Richman is now in her third and final year of internal medicine residency at Stanford University and, next year, she will be Chief Resident there.)

Q5: How do you think that the field will change in the next 5-10 years as technology and knowledge increase?
Dr. Malynn: Initially, I think with the genetics knowledge will be an improvement in diagnosis. Once the correlations get more established you could have more genetic tests in the clinical laboratory to help the clinician decide what the best treatment plan might be for a particular patient. I am not sure how quickly that would come but that is one thing I can see coming before an actual pharmacological intervention which takes much longer to develop. Also, as we continue the basic research sometimes you learn things, for example a pathway that wasn’t thought of for these particular diseases, but you discover it in the research and there may already be a drug available that may be used for other diseases that target the same pathway and it may be worth testing if it is effective in these diseases.

Dr. Ma: Yes, I think it is a very exciting time right now; the technology and biology are much more sophisticated. The information gained from various aspects of the basic science world that have gotten very sophisticated ranging from biochemistry to molecular biology to immunology to understand how molecules and cells function in an increasingly sophisticated fashion and also on a smaller scale makes it more practical to be able to do studies with cells from human samples. It allows us to bring together what we have always thought to be experimental models so that we can now approximate the human condition which brings us closer to the human disease. We should really take advantage of the collaborations between the basic scientists and the clinical scientists which will bring us much closer to using these molecular interventions for therapeutic benefit.
The Laboratory Perspective  

by Kirsten Sterba

Dr. Criswell is very fortunate to collaborate with Dr. Lisa Barcellos and her talented lab team at the UC Berkeley Genetic Epidemiology and Genomics Laboratory. Hong and Diana Quach, the managers of Dr. Barcellos’s lab, process, extract, and store the DNA from the saliva kits collected from all of our RA and lupus study participants. This is no small task because we have collected over 5,000 saliva samples. Processing and extracting DNA from the saliva kits is a lengthy process that takes 5 hours from start to finish. The lab is equipped to extract 48 individual samples per day in 2 separate batches. Once the samples are processed, the lab assigns a unique sample ID to each tube and stores the stock solution in a minus 80 degree Celsius freezer for long term storage, and a “working solution” in a minus 24 degree Celsius freezer for short term storage. Depending on many factors, including age and health status, the amount of usable DNA extracted from each sample varies, but on average the lab is able to extract 100 micrograms from each saliva sample—enough for approximately 100 experiments. But in some cases, the lab is not able to get a viable sample or the yield is low—due to the presence of bacterial DNA or other non-human DNA—for example, if someone forgot to rinse their mouth first before collecting their saliva (all living things have DNA—even apples!). In these cases we need to re-contact participants to obtain an additional sample. Thanks to everyone who has provided extra samples for the study!

In addition to processing and extracting DNA, Dr. Barcellos’ lab team also performs highly technical genotyping experiments for this collaboration, including the new wave in genetics research, called “epigenetics.” (For more details about this, see article written by Dr. Chung). Because of advances in technology, Hong and Diana have had to adapt to the ever-changing lab equipment, reagents, and protocols. Hong and Diana told us that when they first started working in the lab in 2006, they had the ability to genotype 1,500 genes at a time whereas today they are able to genotype millions of genes on a chip as tiny as an iPhone! It is amazing how much progress has been made in the lab in just 6 years!! We are so grateful to collaborate with such a professional and capable lab team!

Keep In Touch!

We want to be able to share future research updates and important news with you about the studies you and your family members are enrolled in. Please call us if:

• Your address or phone number have changed
• You need replacement saliva or blood kits
• You or your family members have developed a new autoimmune disease

Call us toll free at: 1 (888) 223-3067 x 1
Or visit our website: http://medicine.ucsf.edu/lupus

Thank you for your participation in this important research!

Did you know… That philanthropy is moving our research forward? Most of the work you are reading about in this newsletter has been made possible in part by individuals who believe that research into the genetics of these diseases will transform the way patients receive care in the future. If you are interested in learning about opportunities to support our research, please contact:
Dr. Lindsey Criswell, at 415-476-9026, or Lindsey.Criswell@ucsf.edu

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