The ClinSeq® Newsletter

Volume 6, Winter 2013

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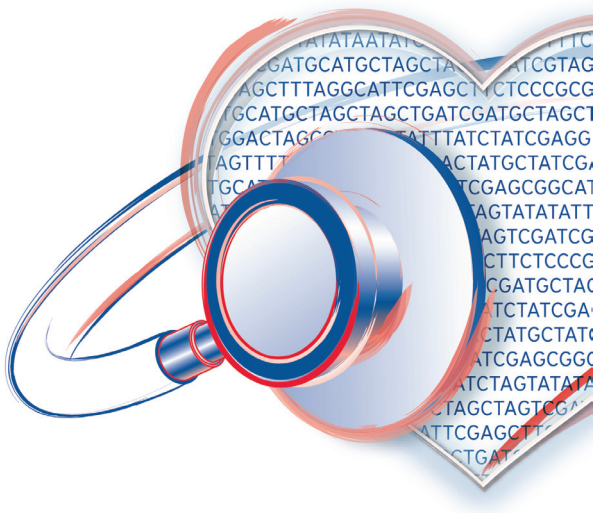
Dear ClinSeq® Participant,

This newsletter will be our fifth installment in news to keep you up to date on the progress of the study. One thing we have learned—a lesson that the entire field is coming to appreciate—is that it is a gigantic amount of work to interpret exomes. The highest impact paper from ClinSeq® in 2012 was the paper authored by Dr. Johnston on cancer gene mutations in ClinSeq® (see below in the list of publications). The data in this paper represent many hundreds of person hours of work to accomplish. Yes, we have the exomes in the computer, but making sense out of them takes longer than generating them. One of the biggest challenges of the field is that there is no 'one stop shopping' for finding data on gene variants. When we come across a variant, we first look in sources like the Human Gene Mutation Database or various gene-specific databases, but these sources are not sufficient to allow us to give a participant a thoughtful and defensible interpretation of a result. To address this, we have to pull scientific papers from the literature and review them manually – and every paper seems to have a slightly different way of doing things and interpreting data. We then have to debate these as a group. Even more challenging is the fact that some of the data sources we look at turn out to be just plain wrong. Sifting through all of this has been a challenge, but we are getting better and faster at it. We are using improved computational tools to speed this process. In fact, ClinSeq® data from the Johnston et al paper were some of the first data to be entered into a huge new database support by the NIH National Center for Biotechnology Information (NCBI – the PubMed and National Library of Medicine people) called "ClinVar" (yes, they copied our style of naming their project and we are happy about it!) This kind of scientific acceleration is essential to get you the results that you are eager to learn. We look forward to working with you and hope that you have a safe, healthy, and joyous 2013.

Leslie G. Biesecker, M.D.
Principal Investigator
Chief, Genetic Disease Research Branch
NHGRI



Contact Information Updates

Are you relocating or changing your phone number? If your phone number(s) or address changes, please let us know. You can call (301) 443-6160 or e-mail clinseq@mail.nih.gov. We need to have your up-to-date contact information so that we can share the latest ClinSeq® information with you and let you know when genetic results become available for you.

Do you have questions about the study or want to refer a participant? If you need information or have questions about your clinical tests (such as your echocardiogram, EKG, CT scan, or blood work) or the study in general, please contact our research assistant at (301) 443-6160.

Community Outreach & Recruitment to Diversify ClinSeq®

At the end of 2011, we had enrolled over 900 participants in the ClinSeq® Project. It is remarkable that over 85% of our participants said their race was white or Caucasian. Having a diverse range of people in ClinSeq® is important to the success of the project, because our findings will be more useful for more people. First, a diverse cohort will enhance our ability to study the role of specific genes in disease. This is because we know that people who come from different racial or ethnic groups may have genetic differences from one another. Those genetic differences may contribute to differing rates of disease between those groups. Second, having a more diverse study group also improves our ability to see whether a person's culture or community affects the impact of their results. This might include whether or how a person shares results with their family, or how they cope with distress after they get a result.

In order to bring a broader range of people to the study, we hired an Outreach Coordinator, Ms. Sandra Epps. More specifically, Ms. Epps is helping us enroll a total of 500 African Americans in ClinSeq®. How does Sandra spend her time? "There has been no typical day so far," she says. One day, she may go to a health fair. The next, she may be working closely with a local community group, such as the District of Columbia Senior Wellness Center. She has also placed our flyers at churches, restaurants, barbershops, and hair salons around the area. Her work has led to a 125% increase in the number of African Americans in our study.

We have received positive feedback from Sandra's outreach efforts. Sandra reports that people often say how important it is for African Americans to join research studies. The most common reasons for joining are to help diversify our findings, and to improve the chances that the findings from ClinSeq® will apply to a broader range of people.

We hope to enroll another 400 African Americans in the ClinSeq® study over the next 2 years. We will share our progress in our newsletters and on our website. If you know someone who may be interested in ClinSeq® or who is hosting a community event where we could recruit, please contact Sandra at 301-402-0020.

Return of Results Update

We receive many calls from our participants each month asking when to expect their genetic results. In our last newsletter, we shared a summary of those results, and received an overwhelmingly positive response to this information. Therefore, we intend to make this a regular feature in our newsletter.

So far, 34 out of our 1,000+ participants have received one or more genetic results. Our current focus is on high-impact genetic alterations that we know or strongly suspect are related to a specific disease or health condition. The results that have been returned are those with the most serious health implications and/or those that we know the most about. Keep in mind that we expect that all participants will be contacted with not just one, but multiple results. Also, it's important to know that just because you have not been contacted does not mean that you do not have an alteration in the genes that we have listed above.

Gene with Variation	Number of Participants with Results Returned	Health Implications
LDLR or APOB	12	High cholesterol at a young age that may require medication
BRCA1 or BRCA2	8	Increased risk for cancers including: breast, ovarian, prostate and others
RYR1	2	Malignant hyperthermia, which causes a fast rise in body temperature and severe muscle contractions after a person is given anesthesia
PMP22	3	Numbness or weakness in the limbs
PKD1	1	Polycystic kidney disease, which causes cysts in the kidney that can lead to high blood pressure and kidney failure
KCNE1, KCNH2, SCN3B, MYH7, PLN	6	Variants associated with heart problems, including abnormalities in heart rhythm and structure
CCR5Delta32	1	Decreased susceptibility to HIV infection, possibly increased susceptibility to West Nile Virus
PPARG	1	Predisposition to abnormal patterns of muscle and fat distribution in the body, and abnormal lab values, such as high triglycerides

Other participants have also been contacted in recent months about genetic variants that have been detected in them that are of unclear clinical significance. These participants are contacted in the context of one of our spinoff studies, where we try to determine the significance of those variants. These studies can lead to new information about whether variants cause disease or not. They can also help us widen the spectrum of phenotypes expected with genetic variations. Thus far, people in this project have been contacted about variants that may cause a variety of conditions, including:

- Atherosclerotic heart disease
- Inflammatory response, which may affect infectious responses as well as risk for heart disease
- Hearing loss
- Vision problems, including glaucoma
- Polycystic liver disease
- Developmental conditions, which may affect how different regions of the body grow and develop
- Laboratory values, ranging from increased calcium in the blood to Protein S deficiency, etc.

Knowledge Amongst ClinSeq® Participants: Summary of a Recent Publication

From January 2009 through May 2011, new ClinSeq® participants were asked to fill out brief surveys at their initial clinic visits. One survey was given before meeting with our genetic counselor to go over the informed consent. A second survey was given after that discussion. Both surveys asked

about many topics, including knowledge of whole exome sequencing (WES) and attitudes toward receiving testing results. Some of the data from these surveys was recently published in a paper titled, "Effects of Informed Consent for Individual Genome Sequencing on Relevant Knowledge"(1). This study aimed to understand whether any factors (such as age or education level) predict a higher level of knowledge about WES. It also aimed to learn whether knowledge changed as a result of the informed consent process. Here, we report on the main findings of that paper.

The surveys asked about knowledge of two aspects of WES. First, there were questions about sequencing limitations, such as the information that scientists cannot learn from WES. There were also questions about sequencing benefits, or the helpful information that may be learned from WES. The research team then looked for differences in knowledge among participants when they were grouped by different traits, including age, education level, and household income. The results in the paper were based on survey responses from 311 ClinSeq® subjects.

The researchers found that several factors were linked with greater pre-consent sequencing limitations knowledge. Those factors included: having more education and higher household income, being non-Hispanic white, and having a lower CAD (coronary artery disease) risk.

More specifically, college graduates were 3.9 times as likely as individuals who had not completed college to have high pre-consent sequencing limitations knowledge. Non-Hispanic whites had higher pre-consent sequencing limitations knowledge at 2.4 times the rate of other racial and ethnic groups. These differences were not seen for sequencing benefits knowledge, which was the same across all groups in the study. This may suggest that people may learn about benefits and limitations in distinct ways. All groups showed greater knowledge following the consent. These findings emphasize the importance of educating study participants so that they have more complete information about both benefits and limitations of WES. With this in mind, we hope to design studies within ClinSeq® in the future that explore new ways of sharing genetic information.

Factors Linked to Increased Pre-Consent Knowledge of Sequencing Limitations
Increased Education
Higher Household Income
Being Non-Hispanic White
Lower CAD Risk

(1) Kaphingst K., et al. Effects of informed consent for individual genome sequencing on relevant knowledge. Clinical Genetics. 82(5): 408-415.

Publications and Presentations

Our research team has been busy sharing what we've learned through our project in the last six months! We've given presentations and posters at a number of professional meetings, including the National Society of Genetic Counselors' Annual Education Conference and the American Society of Human Genetics' Annual Meeting. These include:

- "Gene tests, genome tests and the NIH genetic testing registry." Fisher Center Meeting at Lombardi Comprehensive Cancer Center. Washington DC, 19 Sep 2012.
- "Attitudes toward privacy and personal information sharing among participants in whole-genome research: perspective from two cohorts." National Society of Genetic Counselors Annual Education Conference. Boston, Oct 2012.
- "Loss of function mutations in known human disease genes in 572 exomes." American Society of Human Genetics Annual Meeting. San Francisco, 9 Nov 2012.
- "Challenges in interpreting secondary variants from massively parallel sequencing, perspectives from the ClinSeq® study." American Society of Human Genetics Annual Meeting. San Francisco, 8 Nov 2012.
- "Association of 87 traits related to coronary heart disease and rare sequence variants in the ClinSeq® study." American Society of Human Genetics Annual Meeting. San Francisco, 8 Nov 2012.
- "Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq® study." American Society of Human Genetics Annual Meeting. San Francisco, 9 Nov 2012.
- "ClinSeq®: A pilot study of large-scale medical sequencing in research and implications for clinical genome medicine." American Society of Human Genetics Annual Meeting. San Francisco, 10 Nov 2012.

We have also published the results of several pieces of our study in papers over the last 6 months. We provide a summary of the findings of one of these papers within this newsletter, and hope to share more about others in future editions. Our publications within the last 6 months include:

- Facio FM, Eidem H, Fisher T, Brooks S, Linn A, Kaphingst K, Biesecker LG, Biesecker BB. Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq Study. European Journal of Human Genetics 2012: 1-5
- Chan B, Facio FM, Eidem H, Hull SC, Biesecker LG, Berkman BE. Genomic inheritances: disclosing individual research results from whole-exome sequencing to deceased participants' relatives. Am J Bioeth 2012 12:1-8
- Johnston JJ, Rubinstein WS, Facio FM, Ng D, Singh LN, Teer JK, Mullikin JC, Biesecker LG. Secondary variants in individuals undergoing exome sequencing: screening of

572 individuals identifies high-penetrance mutations in cancer-susceptibility genes. Am J Hum Genet 2012 91:97-108.
- Kaphingst KK, Facio FM, Cheng M-R, Brooks S, Eidem H, Linn A, Biesecker BB, Biesecker LG. Effects of informed consent for individual genome sequencing on relevant knowledge. Clinical Genetics 2012 82:408-415.
- Facio FM, Sapp JC, Linn A, Biesecker LG. Approaches to informed consent for hypothesis-testing and hypothesis-generating clinical genomics research. BMC Med Genomics 2012 5:45.

Enrollment Update

We currently have over 1,000 participants enrolled in the ClinSeq® study. Thank you to everyone who has spoken so highly of the ClinSeq® project and referred others to our study!

Even though the focus of our study has broadened in scope, we are still actively looking for participants who have had a history of heart disease, including those who have had heart attacks, stents, bypass surgery, and high coronary blockage. To be eligible, these interested participants must be between the ages of 45 and 65, live in the metropolitan DC or Baltimore area, have not smoked in the past year, have a primary-care physician, and not have a first-degree relative already participating in the ClinSeq® study. Additionally, we are also actively recruiting African American participants with, or without, a history of heart disease.

Featured Associate Investigator:

Robert Shamburek, M.D.

1) What is your position at NIH?

I am a clinical researcher in the Division of Intramural Research, National Heart, Lung, and Blood Institute. I completed training in internal medicine and gastroenterology and run the Lipid (cholesterol) Clinic in the Cardiovascular and Pulmonary Branch at the Clinical Center. I have followed many families in my clinic with cardiovascular disease and unusual cholesterol levels over the last twenty years in an effort to find the gene responsible for their metabolic disorder.

2) What motivated you to become involved in the ClinSeq® study?

Cardiovascular disease is a complex disorder caused by dietary and environmental factors but is most strongly influenced and regulated by genes involved in the metabolism of cholesterol. The end result of abnormal cholesterol metabolism is often a heart attack or stroke. Over the years, I have followed many families in my clinic who, despite being thin, exercising regularly, and eating a healthy diet, develop cardiovascular disease. Many of these families have increased LDL (bad cholesterol) while others have decreased HDL (good cholesterol). Many of these people have a single mutation (abnormal gene) in a cholesterol transporter, enzyme, or receptor that alters the metabolism of cholesterol. In the past, identifying a single gene mutation was laborious; it often took 10 years.

It is even more perplexing when I find family members who develop cardiovascular disease with relatively normal blood cholesterol levels, suggesting the possibility of completely new causes and defective metabolic pathways. It is clear that there are many more undiscovered cholesterol genes causing heart disease and also many others due to variations in more than one gene. Older genetic techniques are not feasible to look for multiple genes.

The idea behind ClinSeq® is revolutionary for the field of cholesterol because it takes advantage of evolving high-throughput genetic techniques by providing us with the ability to look at multiple genes. It also brought together a group of experts in the fields of genetics, imaging and cardiovascular disease. It is now possible to look at all of a person's genes simultaneously, rather than just one gene, in a fraction of the time and cost. Our dreams have become a reality, but we are now faced with an immensity of data. The analysis of the results is underway, but we are also developing ways to analyze what are just common variations in genes and trying to tease out the potentially causative genes.

The ClinSeq® investigators have been fortunate to have dedicated volunteers in the study who are permitting the analysis and comparisons of their genes, blood, imaging studies, and clinical history. However, the validation of new genes as a cause of cardiovascular disease and the expanding roll of ClinSeq® in other diseases can only be accomplished with additional collaborations. We are only beginning to scratch the surface of the possible disease discoveries that can come out of ClinSeq®, but the future depends on the continued involvement of the volunteers. I continue to be amazed by the interest and motivation of ClinSeq® volunteers when they are called back for additional testing or blood sampling.

3) What are your other research interests?

My research interests focus on the discovery of cholesterol genes that are involved in the development of cardiovascular disease. Our group has followed families who have extremely unusual cholesterol levels (high and low cholesterol or triglyceride or high and low LDL or HDL) that are involved in cardiovascular disease. In some cases, I bring the patients into the hospital and label the cholesterol in their blood to see how it is metabolized on special diets or drugs. These studies allow us to determine whether a defect is due to the overproduction or delayed removal of LDL (bad cholesterol) or HDL (good cholesterol). Our team members can then go back into the laboratory and look for the abnormal metabolic pathways and ultimately the genetic cause of the disorder.