Dear ClinSeq® Participant,

The results of ClinSeq® continue to catalyze change in medicine. I would like to draw attention to a study we have recently published in Clinical Chemistry, which is the leading journal for diagnostic pathology. As trailblazers in clinical genomics, you might not recognize that overall, very few people have been sequenced and very few laboratories offer clinical exome or genome sequencing. It is still novel and laboratory regulatory agencies are rushing to catch up with the science. It turns out that one of the big unknowns is the reliability of results from exome and genome sequencing. Due to a fortunate series of events, ClinSeq® was able to answer one of these key questions.

Before exome sequencing was available, we were actually sequencing the DNA of our first 500 or so participants with an older technology called Sanger sequencing, named after its inventor Frederick Sanger. Sanger sequencing had been in use for decades and was considered the ‘gold standard’ for gene sequencing, mostly because that is all we really had. When exome sequencing came along, it was new and untested, so a de facto standard developed in our field that any gene variant found by exome sequencing had to be confirmed with Sanger sequencing. The challenge is that Sanger is slow and expensive, which is frustrating because exome is fast and inexpensive. Kind of like hooking a donkey cart to an F1 race car. Over the last several years of exome sequencing, we and others were becoming more confident that exome data were very robust – some of us even began to suspect it was superior to Sanger. The ClinSeq® data allowed us to test this. We had, sitting in our computers almost 280,000 Sanger results and tens of millions of exome results – so the question arises as to how useful Sanger is to confirm an exome result.

We tasked one of our trainees to review nearly 5,660 exome variants to see how many of them were confirmed by Sanger. The first result was impressive – 5,641 were confirmed. We next dug deeper and asked why the 19 did not agree and the result was shocking – it turns out that for 17 of the 19, the Sanger ‘confirmation’ was wrong and the original exome result was correct. What this means is that exome findings are 99.965% reliable and that if one does Sanger confirmation, you will spend a lot of money, but worse than that, if you find a discrepancy there are almost 9:1 odds that your “gold standard” is telling you to ignore an exome result, that is in fact correct. This study has begun to have an effect on the clinical diagnostic exome community – which should mean more testing should become available at lower costs, and with better reliability.

This is another example of how your contributions to ClinSeq® are helping to advance the practice of clinical genomics and improve medical care – I can’t tell you how happy I am to be able represent you to the wider community and improve medical practice for all.

Leslie G. Biesecker, M.D.
Principal Investigator
Chief, Genetic Disease Research Branch
NHGRI
Using Genetic Sequence Data to Identify Disease Risk

Jumbled, missing, wrong, and/or extra copies of genetic information can cause improper functioning of a gene. In turn, improper gene function may put someone at a higher risk to develop disease. However, this is not always the case. In many cases, our bodies can tolerate genetic alterations without developing health problems. Researchers on the ClinSeq® study developed a side project to investigate specific types of genetic alterations called “loss of function” variants. These variants are expected to cause health risks by decreasing the normal function of a particular gene. The side project aimed to understand how often these variants lead to signs of related health problems.

Scientists first identified participants whose genetic sequences included these variants. Then, the team used statistics and looked at published data to select variants that were likely to decrease the function of the gene and cause disease risk. These genes are associated with a wide variety of conditions including cancer, glaucoma, or heart defects. Participants with one of those variants were contacted by the study team and offered the opportunity to join this side project. 79 people agreed to enroll in the study.

Each participant in the study then received an evaluation to see if they had a personal or family history of the condition that was related to their genetic variant. Sometimes these evaluations were conducted over the phone, with a researcher asking the participant if they or their family members had certain symptoms of the condition. Other participants returned to the NIH for additional tests, such as MRIs, physical examinations and skin biopsies. In addition to evaluating our participants, we also sometimes involved their family members in the study. We asked those family members to answer questions about their health history and send in saliva samples for DNA testing if they were willing to do so.

Of the 79 participants enrolled in this study, 34 people were positive for signs of the condition in question. This equates to 3% of the overall study population and 43% of those with a loss of function variant in our study who had signs of the condition. 18 out of the 34 participants whose evaluations were positive for the condition in question were not previously aware of the condition. Those participants now have new information about their health, which could be helpful in managing their health and their family members’ health.

In addition to providing health information to our participants, this project also advances our knowledge about the prevalence of genetic conditions. Prior to this study, scientists estimated that 0.02% of people had genetic conditions, but our study data suggest that this number may be 3% or higher. In addition, the findings point to the power of identifying certain kinds of genetic variants in predicting disease risk. Clinicians can use evidence of these variants to guide their evaluations of patients, which is a key component of personalized medicine.

The findings of this study were published in the American Journal of Human Genetics in 2015 and were also featured in the June 4, 2015 edition of the Washington Post.

Randomized Control Trial Wrap-Up

As many of you know, the ClinSeq® study was designed to learn how to best implement genome sequencing. Some of our research takes place in the laboratory and focuses on how to interpret your genetic sequence data. Other research (called “social science”) takes place outside of the lab and focuses on the “human side” of sequencing, such as how you relate to the results you may receive from this study.

“Recently, we completed our first randomized control trial on the best way(s) to return genetic results to participants. The majority of participants who were eligible for the study agreed to receive their results through the study, which allowed us to reach our recruitment goals quickly.”

Thanks to your participation, we have been successful in conducting a series of social science studies to assess participant interest in receiving their results. The chart below shows the breakdown of participant interest in receiving their results.

**Participant Interest in RCT**

- 82% wanted results and came back to NIH
- 16% wanted results, could not come back to NIH
- 2% did not want results/ unable to contact
educational, psychological and practical aspects of learning genetic results. Recently, we completed our first randomized control trial on the best way(s) to return genetic results to participants. This trial has included four different ways of returning results.

In order to complete this trial, we offered results to over 500 ClinSeq® participants. As shown in the graph above, the majority of these participants agreed to participate in the study and returned to the NIH to receive their results. This excellent response rate allowed us to reach our goal of returning results to over 450 participants in just over two years time. It was important that we reach our recruitment goals so that we can draw scientifically significant conclusions from this research and we could not have done it without your help.

Recruitment for this project is over, but our collaborators are beginning the hard work of analyzing the data that we collected. We plan to publish several papers on the results of this trial in the coming years. As usual, participants who enrolled in that trial can get mailed updates with detailed information on those publications and we will provide general updates to everyone through our newsletter.

**Return of Results Update**

We are continuing to review our participants' genetic sequences and to share results with them as they become available. As in previous issues of the newsletter, to the left is a list of the results we have returned to other participants so far.

Please note that all of these results have already been returned. If you have not heard from us, it means that we do not have a result to return to you yet. However, that does not mean that you do not have genetic variants or that we have ruled out your risk for conditions in the table.

<table>
<thead>
<tr>
<th>Gene with Variation</th>
<th>Number of Participants with Results Returned</th>
<th>Health Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR or APOB</td>
<td>13</td>
<td>High cholesterol at a young age that may require medication</td>
</tr>
<tr>
<td>KCNE1, KCNH2, SCN3B, MYH7, PLN, PKP2</td>
<td>11</td>
<td>Variants associated with heart problems, including abnormalities in heart rhythm and structure</td>
</tr>
<tr>
<td>BRCA1, BRCA2, SDHC, MSH6, PMS2</td>
<td>11</td>
<td>Increased risk for various types of cancer</td>
</tr>
<tr>
<td>RYR1</td>
<td>4</td>
<td>Malignant hyperthermia, which causes a fast rise in body temperature and severe muscle contractions after a person is given anesthesia</td>
</tr>
<tr>
<td>PMP22</td>
<td>2</td>
<td>Numbness or weakness in the limbs</td>
</tr>
<tr>
<td>LRRK2</td>
<td>2</td>
<td>Susceptibility to Parkinson's disease</td>
</tr>
<tr>
<td>PKD1</td>
<td>2</td>
<td>Polycystic kidney disease, which causes cysts in the kidney that can lead to high blood pressure and kidney failure</td>
</tr>
<tr>
<td>CCRS5Delta32</td>
<td>1</td>
<td>Increased susceptibility to HIV infection, possibly increased susceptibility to West Nile Virus</td>
</tr>
<tr>
<td>PPARG</td>
<td>1</td>
<td>Predisposition to abnormal patterns of muscle and fat distribution in the body, abnormal lab values, such as high triglycerides</td>
</tr>
<tr>
<td>FLCN</td>
<td>2</td>
<td>Susceptibility to Birt-Hogg-Dube syndrome, which is a condition characterized by benign skin tumors, cancerous or non-cancerous kidney tumors and lung cysts</td>
</tr>
<tr>
<td>SGCE</td>
<td>1</td>
<td>Predisposition to myoclonus-dystonia, which is a condition that causes quick, involuntary muscle jerking or twitching and muscle cramping, such as writer’s cramp</td>
</tr>
<tr>
<td>PROS1</td>
<td>1</td>
<td>Susceptibility to developing abnormal blood clots</td>
</tr>
<tr>
<td>MTND4</td>
<td>1</td>
<td>Susceptibility to an inherited form of vision loss</td>
</tr>
<tr>
<td>SLC4A1</td>
<td>1</td>
<td>A problem with red blood cells that can lead to anemia</td>
</tr>
<tr>
<td>SLCO1B1 and SLCO1B3</td>
<td>9</td>
<td>Increased risk to have side effects from medications prescribed to lower cholesterol</td>
</tr>
<tr>
<td>Various</td>
<td>500</td>
<td>Conditions that are inherited in a specific pattern such that they do not affect your health, but could affect future generations.</td>
</tr>
<tr>
<td>TCAP, DSC2, DES, MYOZ2, LMNA, EYA4, LDB3, TMPO, DTNA, and LAMA4</td>
<td>13</td>
<td>Potential susceptibility to heart disease and other conditions.</td>
</tr>
</tbody>
</table>

Results that are italicized have been returned in the last 6 months.
Farewell from Kristen Fishler

Dear ClinSeq® Participants,

Over the past two years of my research and clinical training at the NIH as a ClinSeq® research assistant, I have learned so much from the ClinSeq® team and our wonderfully enthusiastic participants. I have enjoyed developing relationships with many of you in person and over the phone. Your collective commitment to the field of medicine and curiosity in the future of genomics is a stand out characteristic of this cohort and has been personally inspiring. I take this opportunity to thank each of you for sharing your family histories, words of wisdom, and pleasant demeanors throughout my time with you. I am excited to announce that I plan to expand upon the skills and lessons I have learned as I pursue my dream to attend graduate school for genetic counseling at the University of Cincinnati in Ohio! I am confident that my replacement, Ms. Ilana Miller, will continue to foster the relationships I have created and will break new and exciting ground on this project. I wish you and your families all the best, thank you again for being an important part of my journey!

Warm regards,

Kristen Fishler, BS
ClinSeq® Clinical Research Assistant

Featured Associate Investigator: CDR Steven Gonsalves, PhD, BC-FNP

1. What is your position at the NIH?
I am a clinical nurse practitioner with NIH/ NHGRI on the ClinSeq® study.

2. What motivated you to become involved with the ClinSeq® study?
The opportunity to be a part of a cutting-edge, longitudinal study like ClinSeq®, with its greater ability to discover indicators or predictors of certain illnesses, was a big draw. For my PhD dissertation I analyzed ClinSeq® genetic data and conducted laboratory studies to study a genetic-susceptibility condition known as Malignant Hyperthermia - a potentially fatal, inherited susceptibility disorder associated with the administration of certain types of general anesthetics during medical procedures. Currently, I am interested in the identification and reporting of presumed pathogenic variants for Malignant Hyperthermia in two genes, as well as their impact on drug response, discovered as an incidental or secondary finding from exome sequencing.

3. What are your other research interests?
My other research interests include works to apply current and emerging technology approaches in clinical genomics to understand the impact of specific gene variants on their function and pathogenicity, and to be apart of the efforts to help identify ways to implement sequencing into medical practice.

Enrollment Update

We have recruited 400 participants for our African American, African and Afro-Caribbean cohort with hopes of reaching our goal of 500 individuals before the end of this calendar year. If you know someone of African American, African or Afro-Caribbean descent who is 45-65 year old, has not smoked in the last 12 months, and is willing to participate in our study, please encourage him or her to call our recruitment coordinator, Sandra Epps, at 301-402-0020.

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