Clinical Chemistry

Classifying Germline Sequence Variants in the Era of Next-Generation Sequencing

Article:
Linnea Baudhuin, et al.
Classifying Germline Sequence Variants in the Era of Next-Generation Sequencing.
Clin Chem 2016;62:799-806.
http://www.clinchem.org/content/62/6/799.extract

Guest: Dr. Linnea Baudhuin is Co-Director of the Personalized Genomics Laboratory, Cardiovascular Laboratory Medicine, and the Clinical Genome Sequencing Laboratory at the Mayo Clinic.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Next generation sequencing is increasingly available in clinical laboratories, enabling the sequencing of more genes for relatively lower costs. Laboratories may now offer testing for panels with hundreds of genes, or even characterize whole exomes genomes. Access to this expanse of amount of information has led to advances and diagnosis, prognosis, risk assessment and therapeutic decision making. It is one of the key technologies in precision medicine efforts. However, interpretations of the impact that variations in an individual's DNA will or may have on their health is challenging.

A Q&A in the June 2016 issue of *Clinical Chemistry* features several experts discussing the varied approaches and the tools they use to perform germ line variant interpretation in clinical next generation sequencing. They described their implementation of newly issued guidelines and the associated challenges. We are joined by one of the moderators of that Q&A in this podcast, Dr. Linnea Baudhuin is Co-Director of the Personalized Genomics Laboratory, Cardiovascular Laboratory Medicine, and The Clinical Genome Sequencing Laboratory at the Mayo Clinic.

And Dr. Baudhuin, why did you put together this Q&A article on genetic variants?

Dr. Baudhuin: Well, the impetus for this article has to do with the increasing numbers of laboratories who are getting into the genetic testing space and they are now providing more comprehensive genetic testing due to advances and technology. So many laboratories now perform high-throughput next generation sequencing, or NGS, for hereditary disorders. Thus, new germline panel tests are continually emerging and now even exome and genome sequencing for rare inherited disorders is becoming more widely available. The increasing adoption of NGS testing has really led to a dramatic increase in the number of

© 2016 American Association for Clinical Chemistry

genetic variants that need to be interpreted and reported on.

So what this means for us, the clinical laboratorians, is that we're now being faced with increasing amounts of genomic data that we must translate into meaningful clinical reports. There's really a wide variety of depths, experience, and expertise in germline variant classification, both within and between laboratories. Furthermore, it is quite difficult to apply a completely standardized approach to genetic variant classification, because there are only certain aspects of this that can be standardized; whereas the remainder of classification efforts must rely on the practice of laboratory medicine, which includes individual laboratory, internal standards and rules for variant classification. Therefore, we decided to put this article together so that we could ask our expert colleagues about their own methods for genetic variant data curation and comparison and classification. We really wanted to learn from them as well as highlight some of the important complexities that are out there.

Bob Barrett: Doctor, what are germline sequence variants and why are they sometimes challenging to classify?

Dr. Baudhuin: Germline sequence variants are genetic variants that can be passed on from one generation to the next. They can sometimes cause hereditary disorders. For example, you might think of cystic fibrosis, some familial cancer syndromes, and some cardiovascular genetic disorders like the cardiomyopathies. As far as how we classify germline sequence variants, we can categorize them into multiple different ways depending on their causative relationship with the disorder in question. And probably the most common classification system involve the five tier system with categories of pathogenic, likely pathogenic, variant of uncertain significance, likely benign, and benign. There are additional classification systems that some laboratories use that include additional tiers, which most of them give additional stratification to the variant of uncertain significance category.

You asked about why germline sequence variants are sometimes challenging to classify. Sometimes it's actually quite a straight forward process to classify germline sequence variants, but we often encounter variants that we can't easily put into one of those categories of pathogenic or likely pathogenic, et cetera. In these cases, we can utilize as many tools and guidelines as possible as a starting point, but we ultimately have to rely on our own internal knowledge and judgment to classify the variant. And there are so many different elements that we have to take into consideration like disease prevalence, variant frequency in patient and control populations, the quality of the population

© 2016 American Association for Clinical Chemistry

variant data that we're looking at, ethnicity considerations, gene function and so on. We also really rely heavily in some cases on the patient phenotypic information that's provided to us when the test is ordered.

However, often times many of these variables that I've mentioned are incomplete or poorly understood and so then this can exacerbate the challenge as a variant classification.

Bob Barrett: And why is accurately classifying genetic variant so important?

Dr. Baudhuin: Well it's so important to accurately classify genetic variants because of the implications to patient diagnosis and management, as well as follow-up testing and management of family members.

So, let me run you through an example scenario. Let's say, we identify a variant in a patient with Long QT Syndrome and we classify the variant as likely pathogenic, so likely to be causative for that patient's Long QT Syndrome. The patient has several children and they're all screened for the variant, and then eventually extended family members such as siblings and cousins are also screened for the variant. Well, each family member would be expected to have appropriate follow up depending on whether or not they harbor the variant. For example, those that test positive should receive electrocardiograms, appropriate drug therapy, and likely more often than not, they would receive an implantable cardioverter defibrillator or a pacemaker. Those that test negative for the variant would be assumed to be at low risk for sudden cardiac death due to Long QT Syndrome, so they wouldn't have any further follow up or procedures or drug therapies.

Now let's say down the line, we take another look at that variant and there's more data available related to it, and we determine that, oops, that variant is not likely pathogenic anymore, it's actually likely benign. So what happens with all those family members? All of those that tested positive may need to have their pacemakers removed, and all the families who tested negative will need to be brought back in for all those follow-up things like electrocardiograms and other testing. And then there's also the possibility that in the meantime, one of the family members who tested negative and was presumed free of risk had a sudden cardiac event. And as you can imagine, there are many other scenarios like this depending on how a variant is classified and which gene is involved and which disease state is involved. So therefore, anytime we report a variant, we need to be extremely careful because there are so many patient and family clinical care implications.

© 2016 American Association for Clinical Chemistry

Bob Barrett: Are there some guidelines or tools or anything else in the bag of tricks that can help with the challenges of classifying variants?

Dr. Baudhuin: Well yes actually, last year a group of stakeholders from several prominent organizations including the American College of Medical Genetics and Genomics, the Association for Molecular Pathology, and the College of American Pathologists, published guidelines that provided a revised and expanded framework for classifying germline sequence variants. And this has been extremely helpful to the clinical community. Additionally, another resource has been developed and provided. It's a publically available database provided by the clinical genome resource, and this database is known as ClinVar. ClinVar is really helpful because it stores both individual and consensus variant classification information that's been put into the database by different laboratories.

Additionally, with the explosion in the technology and it being so much cheaper, we now have these really fantastic population variant frequency databases. Most of these are stratified by ethnicity which is really helpful, and include thousands of exomes. These databases have been put together by groups such as the Exome Sequencing Project group and the Exome Aggregation Consortium group. However, while these strides have definitely been helpful, there are still many limitations to the guidelines and databases and they're definitely not a one-stop shop. In terms of additional tools available to help with variant classification, we specifically thought this information from expert laboratory directors in our Q&A. And what we found out is that most laboratories have to rely on their own in-house developed tools to assist with any type of automation of variant classification, or even other aspects of variant classification like pulling in data from all of these databases and other resources.

Really for inherited disorders, they're just aren't very many commercial tools out there that can compile all the different sources of information into a robust manner, and integrate with the laboratory information and reporting systems. That's really a complex and such an evolving area. There are so many different nuances to each LIF and the sources of information and data are constantly changing. So it really falls upon the laboratories to build these tools and this can be a very expensive and daunting process. And we have to try to keep up with everything that's out there.

While automating these tools and building them really does benefit in streamline the laboratory workflow, building in that automation and building these tools can become very

© 2016 American Association for Clinical Chemistry

complicated quite quickly and it can become outdated quite quickly.

Bob Barrett: What were some of the things that surprised you the most about the responses of the experts in your Q&A?

Dr. Baudhuin: Well, in regards to those guidelines that I just mentioned, the ACMG, AMT, CAP guidelines that were published in 2015, we weren't really sure what to expect in terms of how well those guidelines have been received by other laboratories, but the feedback that we got from the experts is that they really widely embraced the guidelines and utilized them. They all agreed that the guidelines were thoughtfully developed. They provide a measure of uniformity to variant assessment and they can be especially helpful in the decision making processes for variants that are really difficult to classify. They also mentioned that the guidelines can at least partially enable automation of variant assessment. But it was noted that the guidelines are more useful for some genes and disorders as compared to others. For example for some disorders such as rare pediatric genetic disorders, the guidelines have been found to be too conservative, and it was also recognized that for variants that are encountered in genes without established disease association such as what we often encounter with exome sequencing that the guidelines really are not as helpful. But I think overall what the expert said is that yes, the guidelines are very useful as a baseline decision-making guide for most variants. But there are always exceptions and ultimately as I've stated, the laboratory team has to rely on its own best judgment to classify a particular variant.

We were also pleasantly surprised to note how evolved some laboratories are in terms of databases and automation of variant interpretation and we are very reassured by the team approach that many laboratories utilize for variant classification and it was reassuring that the experts recognize the tremendous power and benefit as well as the limitations of ClinVar, the database that I described earlier. In terms of ClinVar, it's really a fantastic resource for determining how other laboratories have classified variants, and what type of consensus there is, but the experts noted that not all laboratories serve are reliable source of variant classification. There are some challenges in depositing data into ClinVar. So what this means is that the ClinVar database is likely missing a lot of data that could be there if laboratories had a more facile way to upload their data. Therefore, the database can be a great starting point but absolutely has some opportunity for growth.

Another area that we were surprised about, or interested in, in terms of the response of the experts and the Q&A is that

© 2016 American Association for Clinical Chemistry

each laboratory has quite different policies towards what it uses as a minor allele population frequency cutoff for classification of variants as benign or likely benign. And this is really an area that I think we need to come together as a community and really develop some guidelines on, because for years, there's been a somewhat arbitrary cutoff of variants having greater than one percent minor allele frequency in the population as being considered as benign. However, now we have all this great population frequency information available to us in some of the databases that I mentioned already like ESP and ExAc, and it's really becoming more apparent that the cutoff can probably be lower since pathogenic variants for most rare disorders are present at a very, very low frequency and it is quite unusual to have common rare variants—yes, I meant to say common rar—that would account for most of the disease. This is really uncharted territory, as I said, and so it would be great for us to come together as a community and develop some consensus for standard cutoff as well as disease specific cutoff.

Bob Barrett: Okay. Well before we wrap this up, do you have any final takeaway messages that you can share about variant classification for hereditary disorders in the current area of high-throughput genetic testing?

Dr. Baudhuin: Yes I do, and it really is truly refreshing to see how many strides we've made as a community towards providing consensus guidelines and variant classification, towards sharing data, and moving towards the common good of providing optimal patient care. Because of the nuances of variant classification, including disease and gene specificity, it is so important that we have a team approach bringing in clinical experts when necessary to provide the best possible understanding of the potential relationship of variants to specific phenotypes.

And with the team approach, we can properly vet the novel variants that we detect and provide really well thought out variant interpretations and patient reports. Ultimately, the goal is to provide clear and accurate variant interpretations to guide patient diagnoses and management, ultimately being able to use genetic results to perform cascade testing on family members when appropriate. And we're certainly in exciting times with the advances in genetic testing technology, and we hope to utilize these tools to the best of our ability to help advance personalized medicine and improve the diagnosis, care, and management of patients and their families.

Bob Barrett: Dr. Linnea Baudhuin is Co-Director of the Personalized Genomics Laboratory, Cardiovascular Laboratory Medicine, and the Clinical Genome Sequencing Laboratory at the Mayo

© 2016 American Association for Clinical Chemistry

Clinic. She's been our guest in this podcast from *Clinical Chemistry.*

I'm Bob Barrett. Thanks for listening.

© 2016 American Association for Clinical Chemistry