Clinical Chemistry

A Mendelian Randomization-Based Approach to Identify Early and Sensitive Diagnostic Biomarkers of Disease

Article:
Pedrum Mohammadi-Shemirani, et al.
A Mendelian Randomization-Based Approach to Identify Early and Sensitive Diagnostic Biomarkers of Disease
Clin Chem 2019;65:427-36.
http://clinchem.aaccjnls.org/content/65/3/427
Guests: Drs. Pedrum Mohammadi-Shemirani and Guillaume Paré of McMaster University in Hamilton, Ontario, Canada.

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

Identifying markers of chronic kidney disease that occur early in the disease process and are specific to loss of kidney function may allow timely more accurate identification of patients who will eventually develop the disease.

In the March 2019 issue of *Clinical Chemistry*, a paper investigated potential blood markers of early chronic kidney disease which are caused by loss of kidney function using an innovative reverse Mendelian randomization approach. Mendelian randomization is a genetic epidemiological approach that is made substantial inroads into our understanding of the causes and consequences of disease.

Two of the authors of that paper are Pedrum Mohammadi-Shemirani and Guillaume Paré. Dr. Paré is Associate Professor and University Scholar in the Department of Pathology and Molecular Medicine at McMaster University in Hamilton, Ontario, in Canada, where he also serves as Director of Genetic and Molecular Epidemiology at the Laboratory Population Health Research Institute.

Pedrum Mohammadi-Shemirani is a doctoral student of Professor Paré and specializes in bioinformatics research with a focus on applying genomics and molecular data to better understand disease and improve patient care and outcomes. They are both our guests in this podcast, and Dr. Paré, we'll start with you.

Before we delve into reverse Mendelian randomization, what exactly is the rationale behind the technique of Mendelian randomization? How is it used in epidemiology?

Dr. Paré: So, Mendelian randomization is a statistical genetic technique to infer causal relationship between risk factors and outcome of interest. And it relies on the second law of Mendel, which is the random allocation of alleles at meiosis, which essentially states that alleles are randomly allocated

at meiosis and this randomization is very useful, and it's useful for two reasons.

First of all is that it is immune to some of the confounding that we can find in epidemiological studies such as, let's say, the environment or other exposures that have nothing to do with the disease of interest. And the second reason why this is very useful is because it's not immune to reverse causation. So, genetic variants can lead to a disease. But generally speaking, a disease will not lead to a genetic variant.

And essentially, why this is so useful and getting so popular is that we can then test hypothesis on whether, for example, LDL cholesterol is causally related to cardiovascular disease. And in this case, both Mendelian randomization and clinical trials are telling us that, "yes." And this is very important because this is not always the case for the risk factor outcome association that we see. A good way to conceptualize Mendelian randomization is to look at the parallel between Mendelian randomization and a randomized clinical trial.

So, in a randomized clinical trial, an investigator will randomize participants to either an active drug or placebo. And then, after a few years of follow up, we will see the effect of this drug, for example, on blood cholesterol, and we will see the effect of this drug on cardiovascular outcomes, and we will decide whether this drug is efficacious or not at decreasing cardiovascular disease.

Mendelian randomization has many parallels except that, in this case, it's not an investigator that's randomizing individuals due to drug or not, it's the second law of Mendel that is randomizing individuals to have alleles that either increases a little bit cholesterol or decreases cholesterol by a tiny bit. And then, after a follow up, we see if people with the cholesterol-raising allele developed more cardiovascular disease.

Some of the key differences here is that in a drug trial, usually the effect on the risk factor is quite pronounced. When we think in terms of genetic effects, these genetic effects are usually tiny. But on the other hand, we have a lifelong follow-up of a genetic exposure. So, people are born with an allele that gives them slightly higher cholesterol, whereas in a randomized clinical trial, the follow up is limited by the length of follow-up of that trial.

Bob Barrett: And how did the idea of reverse Mendelian randomization come about? Why would that be important to the context of identifying diagnostic biomarkers?

Dr. Shemirani: So, for the first part, Mendelian randomization is traditionally applied in the context of identifying a biomarker that causes a disease. So, these biomarkers would be, for example, promising targets for pharmaceutical interventions, and this has been shown in the context of cardiovascular disease.

For example, PCSK9 inhibitors, statins, and more recently, ACLY, have all been validated with Mendelian randomization to lower LDL cholesterol and decrease the risk of coronary artery disease, which has also been shown in corresponding randomized control trials.

And so, it's kind of a natural extension to apply these principles of Mendelian randomization in the opposite direction to instead investigate, "Does a disease cause any biomarkers to be elevated or decreased?" And this question is better suited to addressing whether a biomarker would be a promising diagnostic candidate rather than a pharmaceutical intervention.

So, to answer the second part of that question, "Why is causality important?", causality is important because a causal diagnostic biomarker would, in theory, always be a consequence of the disease, and therefore be more sensitive as well as being expressed earlier in the pathogenesis of the disease. For instance, in the context of CKD, an observational study may find several serum biomarkers elevated in CKD cases relative to controls. However, some of these biomarkers may be due to an earlier cause of CKD like diabetes or BMI rather than CKD itself.

Another useful aspect of this reverse Mendelian randomization is investigating whether these biomarkers that maybe are identified would be caused by the disease or we can investigate to see whether they are indeed caused by diabetes, or obesity, or any other common set of risk factors for the disease of interest.

Dr. Paré: I would say that, essentially, the promise of reverse Mendelian randomization is to identify biomarkers that are both more sensitive and more specific to disease. Sensitive because they can detect early disease though these genetic associations, but specific because we can also test for potential confounders or related risk factors and to make sure that they do not impact the biomarker of interest.

Bob Barrett: Okay. Now, in your paper in *Clinical Chemistry*, you applied that technique to kidney function and chronic kidney disease. Why did you choose that area of disease and diagnosis?

Dr. Paré: So, this is an excellent question and I think you know our paper can be seen as a proof of concept here on applying Mendelian randomization to biomarker discovery. We thought that CKD was a particularly good example for two reasons.

The first reason, which is really the most important one is that there is a clinical need for earlier markers of CKD. Unfortunately, we do know that carotenemia is relatively insensitive to early changes in kidney function. And likewise, albuminuria can be considered as a later marker of kidney damage.

On the other hand, we do have interventions that are efficacious at decreasing progression of kidney disease. And therefore, there has been unmet medical need to have earlier markers of chronic kidney disease so we can identify individuals with very early, very subtle decrease in kidney function, and we could have interventions to stymie the loss of kidney function in these individuals.

The second reason is that, we also add a lot of genetic data that gives us the primary material to be able to apply this method. In fact, there are many very large international consortia that have looked at the genetics of eGFR and CKD, and we really need to have these data to be able to apply reverse Mendelian randomization.

So, in effect, there was a little bit of a perfect storm between something that we thought is clinically useful and for which we thought we had everything necessary to conduct the analysis.

Bob Barrett: Your results suggest that protein trefoil factor 3 may be a valuable diagnostic marker for early chronic kidney disease. What is known about that protein and its potential role in chronic kidney disease?

Dr. Shemirani: Yeah. So, trefoil factor 3 is actually a part of a broader family of trefoil factor proteins, which also include trefoil factor 1 and trefoil factor 2. And their biological role is largely unclear, currently.

There is some literature that has investigated the role in terms of colorectal cancers and other diseases, but there have also been several smaller observational studies that we have cited in our paper that identified increased trefoil factor 3 to be predictive of decreased eGFR in both urine and serum samples. And the prevailing thought is that trefoil factor 3 is a protein that's involved in cellular repair and cellular restitution, which also makes sense in the context of our data since we found that increased trefoil factor 3 is the cause of chronic kidney disease and decrease to GFR, which

suggests that the kidney perhaps may be attempting to repair damage that is being done over the course of this disease.

Bob Barrett: Well, finally, let's look ahead. There must be other diseases where you can apply reverse Mendelian randomization. Where do we go from here? Do you have some prime candidates?

Dr. Paré: Yes, absolutely. I think we have shown in this report that reverse Mendelian randomization can be used to identify markers of disease. And I think there are many more diseases where we could have improved blood biomarkers. One of them that we're particularly interested in is early cognitive decline, which is a terrible disease and I think that adding early markers would be useful. And I think we can think about the whole host of latency disease where we could intervene in early stages that we could see benefit. And I think cancer comes to mind as well.

And I think, clearly, with data accumulating rapidly, both genetic data that is necessary to apply these techniques but also with proteomics data and metabolomics, we hope to be able to apply them to not only more disease but more comprehensive set of biomarkers to try to find like the very best biomarkers for each of these diseases.

Bob Barrett: That was Dr. Guillaume Paré in the Department of Pathology and Molecular Medicine at McMaster University, Hamilton, Ontario in Canada. He was joined by his co-author, Pedrum Mohammadi-Shemirani, a doctoral student of Professor Paré. They have both been our guests in this podcast on potential early markers of chronic kidney disease uncovered by reverse Mendelian randomization. Their paper appears in the March 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!