

**Article:**

Ann M Moyer, Emma Lundquist, Emily K Thoreson, Michelle L Kluge, Katrina E Kotzer, Elyse M Love, Heather L Glessner, Sarah K Arneson, Loralie J Langman, Linnea M Baudhuin, John Logan Black.

DPYD Sequencing Identifies More Clinically Relevant Variants as Compared to Targeted Genotyping.

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Guest: Dr. Ann Moyer is a professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, MN.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

Fluoropyrimidines are widely used to treat a variety of solid tumors, but these compounds carry a substantial risk of severe toxicity or even death. Patients at greatest risk have a deficiency in DPD, the enzyme that metabolizes these drugs. Fortunately, these patients can be identified before initiating treatment by testing for genetic variants associated with enzyme deficiency, allowing physicians to reduce the dose or select a different drug altogether. Sounds simple, right? It's not.

Hundreds of variants have been identified in *DPYD*, the gene that encodes the DPD enzyme, but only four of these are included in the core panel recommended for all populations. Most clinical laboratories performing *DPYD* testing evaluate only these targeted variants, which will detect most patients with deficient DPD activity, but definitely not all. Is there a better way to find these patients with rare *DPYD* variants? Is sequencing the whole gene clearly a better alternative or does that simply trade one set of problems for another?

A research article in the June 2026 issue of *Clinical Chemistry* evaluates nine years' worth of *DPYD* sequencing results, determines the additional yield of sequencing relative to targeted testing, and discusses challenges unique to the sequencing approach. Today, we'll speak with the article's lead author. Dr. Ann Moyer is a professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota. She is a molecular genetic pathologist specializing in pharmacogenomics, renal genetics, and inborn errors of immunity. And Dr. Moyer, let's start with a few questions. What is *DPYD* and why is this testing performed, and, well, how do most laboratories perform this test today?

Ann Moyer:

That's a fantastic question. So, *DPYD* is a gene that everybody's been talking about lately, and the reason for that is a lot of patients end up being prescribed drugs called

fluoropyrimidines that are used pretty frequently for solid tumors. So, those might include colorectal cancer, breast cancer, and other solid tumors.

So, *DPYD* is a gene and it encodes an enzyme called dihydropyrimidine dehydrogenase, which is kind of a mouthful, so we usually just call it DPD for short, and that enzyme metabolizes the bulk of the 5-fluorouracil or fluoropyrimidine that would be prescribed, so that's about like 80% of the dose gets metabolized to something inactive by this particular enzyme. And so, usually, this testing would be performed prior to giving someone that chemotherapy so that you can identify which patients might be at higher risk for toxicity because if they have a genetic variant in *DPYD*, they might have a lower enzyme activity and then they won't be able to metabolize and eliminate as much of that drug, and then that's what leads to the toxicity.

So, what laboratories are doing today is they're generally doing genotyping. And so, that means that basically you're looking at the *DPYD* gene, you're looking for very specific variants and trying to identify who might be at risk for toxicity based on the results of that genetic testing.

Bob Barrett: If most labs are performing genotyping, why was sequencing performed in this study? What's the difference?

Ann Moyer: So that's another great question. So, for the most part, laboratories like to do genotyping for *DPYD* because it tends to have a quicker turnaround time. It doesn't take us long to get a result for a patient and it's less expensive. And at this point in time, the field has really worked on harmonizing and coming together and identifying, well, which variants should be included in one of these genotyping tests.

So, the Association for Molecular Pathology [AMP] has teamed up with a number of other professional societies, and, in general, they've put together this recommendation of a Tier 1 set of alleles and a Tier 2 set of alleles. But, the issue is that we still don't really entirely know what all genetic variants might be present in other populations. And even if we know which variants we're calling Tier 1 and Tier 2, those tend to be the ones that are most commonly identified in patients, but there are a lot of patients out there that happen to have rare variants in *DPYD*, and those could also lead to toxicity. And if you're only genotyping for these specific variants that you're looking for in your test, you're not going to be able to identify those rare variants.

And so, our goal was we really wanted to do a study to get a better understanding of should we be sequencing everybody? Is it okay that we're genotyping patients? And, how often do we find these rare variants that could potentially be clinically

significant? And so, since our laboratory's actually been doing sequencing for a long time because we're also interested in having a test that would work for the hereditary DPD deficiency that has a wide variety of phenotypes that tend to be a little bit more on the neurological side. We already had the test, so we were able to leverage the data that we had from sequencing to start to better understand what we would be missing with genotyping and how many additional variants we could identify with our sequencing test. And so, that was really the goal. And just in general, we found that indeed, a lot of patients do have these rare variants.

Bob Barrett: So, what was the outcome of the study?

Ann Moyer: Well, we ended up finding that it seemed like for the most part, if you're doing genotyping, you're going to find the bulk of patients that happen to have a variant that could impact DPD activity and may predict risk of toxicity. So, in our study, if we looked at just four variants that were in the original Clinical Pharmacogenetics Implementation Consortium, or CPIC, guideline, in our cohort of about 7,000 patients, we ended up with about 8.67% had a variant when you're just looking at those four. But if you expanded that to the AMP Tier 1 variants, it was about 9.3%. And if you went all the way to sequencing and you only included pathogenic and likely pathogenic variants, it was about 10% of patients in our cohort that had a variant. And if you expanded to all of the patients who could potentially have a variant that might be clinically impactful, so a variant of uncertain significance where we're not 100% sure if that would lead to toxicity or if it's actually something benign, it was about a little under 13%, 12.7% that ended up having a variant that we identified.

So, as you can see, even if you're genotyping, you're really going to identify a lot of patients with these important genetic variants, but as you move to sequencing, you can potentially pick up more patients that would have been missed that are also still at risk for toxicity and then they may be able to get a dose reduction.

Bob Barrett: Dr. Moyer, if a laboratory wanted to implement *DPYD* sequencing, what challenges may they encounter?

Ann Moyer: I think the biggest challenge ends up being that, if you decide you want to do sequencing, is how do you handle those variants of uncertain significance and what do you do exactly when you find a variant that hasn't been described in the CPIC expert panel already. So, how the variant classification usually happens today in pharmacogenomics is that CPIC, or the Clinical Pharmacogenetics Implementation Consortium, they have an expert panel that looks at each gene and then they look at the variants that we know about already, and

then they're able to go through and as a group make some determinations about what they think the clinical significance of that would be.

But, if you're a laboratory and you're doing sequencing, it's entirely possible that you'll find variants that are rare enough that the expert panel hasn't seen them yet. And what's really hard is that laboratories today don't have a framework that they can use for pharmacogenomics to classify variants that's widely adopted and standardized across laboratories. So, like when we do inherited disease testing, we've got the ACMG [American College of Medical Genetics and Genomics] and AMP guidelines that we can use. And for pharmacogenomics, they somewhat work, but you really have to adapt them a little bit.

And so, I think that's probably the biggest challenge that laboratories would encounter is they just need to be able to be prepared for how exactly they will be classifying those variants as they encounter them when they haven't previously been classified by another group. But the other thing that tends to be a little bit tricky with sequencing rather than genotyping is, as I mentioned before, a lot of labs are doing genotyping today because it tends to be less expensive and have a quicker turnaround time.

And so, just making sure that the clinicians that would be ordering your test know what to expect in terms of how long it'll take to get a result and what the additional cost may be if they're used to genotyping. But, in general, I think there's a lot of demand right now because a lot of like the FDA labels have changed recently and clinicians are very widely adopting, doing this testing up front prior to giving drugs. So, I think you'll be hearing more requests than you have historically.

Bob Barrett: Well, finally, Doctor, in the paper, you mentioned several discrepancies between the CPIC classification and your laboratory's classification. Why are there differences?

Ann Moyer: So, this was something that we hadn't really thought that much about before, and as we started to look at some of our variants, we realized that the approaches were a little bit different. So, what our laboratory has historically done is we use the ACMG guidelines because that's what we're used to using for inherited disorders, and then we end up basically adjusting them a little bit for pharmacogenomics because some of the criteria don't necessarily completely apply. Like, for example, when you're looking at inherited disorders, if you've got a very common genetic variant in the general population, it's probably not causing disease, but in terms of pharmacogenomics, there may not be selection against that, so you might be able to tolerate higher allele frequencies.

So, just in general, we have to make some adjustments for that. And again, we're used to doing that because we do a lot of diagnostic testing for inherited disease as well. But when we were looking into it more when we found a couple of variants where we didn't necessarily agree with CPIC and were trying to think, well, why is that, we realized, well, the way that they actually classify variants is a little bit different beyond just having an expert panel.

So, the ACMG system really is looking at, well, how likely is this variant to cause disease, but it doesn't necessarily think about the size of the effect. So, since we're talking about an enzyme, like how much is that decrease in enzyme activity, that's not really part of that system. But when you're looking at what CPIC is doing, they're classifying their variants based on, oh, this is a normal function allele, it's a no function allele, or maybe it's in between and it's a reduced function allele. And then, they have a separate term where they apply how likely they think it is to actually have that function. So, they have some variants that might have strong evidence while others might have limited evidence.

Well, it turns out that the things that we wanted to call variants of uncertain significance, or VUSs, using the ACMG adapted system for what we've been doing for pharmacogenomics is really very similar to what CPIC is calling limited evidence. And so, that's really kind of where some of the differences are. And so, it was something that as we thought about it more and reflected on it, we thought it was really interesting in that for enzymes as a whole, it might be helpful even thinking about the ACMG system to be able to incorporate in at some point in time the effect size of some of these changes and what they actually do to the encoded protein.

And so, just in general, though, at the end of the day, even if everybody was using the exact same method, sometimes you can still have some differences of opinion because when you're doing variant classification, clinically, it does require a lot of professional expertise and professional judgment. And so, you can sometimes have those subtle differences. But really, just the approaches are a lot more different than I had originally appreciated despite having been in the pharmacogenomics field for a long time.

And so, I think that's just something for laboratories to be aware of, especially if you're planning to do more sequencing in the pharmacogenomics space. I think the good news is that I think over time, the pharmacogenomics community has been partnering a little bit more with ClinGen and so, maybe over time, we'll be able to see some frameworks in place that

are officially standardized and useful across laboratories for classifying PGx [pharmacogenomic] variants, too.

Bob Barrett:

That was Dr. Ann Moyer from Mayo Clinic in Rochester, Minnesota. She wrote a research article in the June 2026 issue of *Clinical Chemistry* showing increased *DPYD* variant detection through gene sequencing and she's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.