



Your genes on drugs: context matters! Episode 3 Transcript and Show Notes

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Facilitated by the Atlas of Variant Effect Alliance

Alex Nguyen Ba: Welcome back to the VUS podcast, a series initiated by the outreach efforts of the Atlas of Variant Effects alliance. I'm Alex, a professor at the University of Toronto, and together with Evelina, Kortni, Adrine, and Moez, we wanted to reflect on the current advances in the field of genetics.

Our goal is to advocate for increased awareness of rare genetic variants and genetic diseases by interviewing researchers in clinical genetics. To do so, we're going to examine the intersection of rare disease genetics and high-throughput functional genomics.

This podcast focuses primarily on the work of testing variant effects on a very large scale, and within our research we are working to expand this Atlas of Variant Effects for all human genes in the hope that this Atlas will lead to better patient outcomes.

In this episode, we're going to ask ourselves, do our genes dictate which drugs are prescribed to us in case of diseases? What exactly is precision medicine? Is that just a buzzword? Listen on as we interview world leaders in this field.

Finally, we would like to note that this podcast focuses on a realistic portrayal of the basic research behind rare diseases with the hope that it will lead to better patient care, but this podcast is not meant to be medical advice.

[Intro music plays]

Evelina Tronina: Hi, I'm Evelina.

Kortni Kindree: And I'm Kortni, in this scientific series we have focused on the need to test variants in large-scale and the applications of variant testing in disease. Now we climb one more step in complexity and explore the intersection between medication response and genetics. When you are sick and take medication, before your symptoms go away, the drug needs to be absorbed, processed and distributed. The study of this field is called pharmacokinetics.

Evelina: Another name for this field is pharmacodynamics. Basically, pharmacokinetics is what the body does to the medication, and pharmacodynamics is what the medication does to the body. Surprisingly, or maybe not, your genetic makeup may change these processes drastically. Today we talked to leading specialists in this field. To discover how your genes can change your drug response. Let's start from the beginning, from fetal development.

Kortni: So, it's common knowledge that pregnant people or people wishing to conceive should take supplements like folate, but what is not completely understood is that there is a gene that controls folate metabolism. Mutations in this gene can increase the risk for neural tube defects in the growing person.

Evelina: We spoke to Dr. Fritz Roth, a researcher that led a deep mutational scan of the *MTHFR* gene, responsible for folate metabolism. By the way, we explored deep mutational scanning experiments in episode 1.

Kortni: Hello Dr. Roth! Welcome to the podcast! Can you please introduce yourself to our listeners?

Fritz Roth: Yeah, hi, I'm Fritz Roth and I'm primarily affiliated with the University of Pittsburgh School of Medicine. I'm a professor and chair in the Department of Computational and Systems Biology. I'm also appointed at the University of Toronto and at Sinai Health in Toronto where I'm wrapping up a couple of labs there and transitioning here to Pittsburgh.

Kortni: So, what got you to be interested in *MTHFR*?

Fritz Roth: Well, I was at a meeting where Dr. Stan Fields talked about some of his work on *BRCA1* and I thought this was super cool. And at the very same meeting, Jasper Ryan, who was actually my undergraduate advisor when I was at UC Berkeley gave a talk about *MTHFR* and how *MTHFR* was interesting. I got inspired by those two talks to get into MAVEs, and then I had *MTHFR* on my mind.

Evelina: If you recall, MAVEs are “Multiplexed Assays for Variant Effects”.

Kortni: So this gene is important for folate metabolism and neural development, what makes it so interesting?

Fritz Roth: One thing that makes *MTHFR* super interesting is that it has a variant in it, A222V, or alanine at position 222 is changed to valine, which is super common. It's 30% minor allele frequency worldwide. And that means half of us are carriers of this and 10% of us have both copies of this gene that have this variant. And so that means a lot of people when they get prenatal genetic testing will get a result that concerns them. And if you do 23andme, you might be told you're heterozygous or 10% of us homozygous, have both copies with this variant. And so you have a slightly elevated level of homocysteine at normal dietary folate levels. And you can actually bring it down by having a higher level of folate and it's not a problem.

Kortni: Well what happens otherwise?

Fritz Roth: If you're pregnant and don't get the doctor recommended level of folate, then there's an increased risk of birth defects. And so it is important, it is serious but easily remedied, especially if you know about it. It's super interesting because the effects of deficiency in the gene depend on the environment. And this is a fundamental question in genetics: why do many people with the same variant have different outcomes? And you know, this is called incomplete penetrance or if it's a quantitative trait, it's variable expressivity. Those are just words, but basically, why is it that some people have the variant and get a disease and some people don't and sometimes it's an accident of development that made you more prone to a condition. Or its environment and environment can be broadly considered as diet or exposure to pathogens or exposure to toxins. And it can also be due to genetic interactions.

Fritz Roth: That is to say, if each of us have a different genetic makeup at the other genes besides *MTHFR*, then is it a variant at some other position that's making you more susceptible to this damaging variant of *MTHFR*? So that got me excited, not only to work on variant effects and

test them at large scale, but that there was such depth to this problem, this was a gene where environment mattered.

Kortni: Wow, then disease risk is way more complex than we thought. So then this means that there may also be an interaction between the A222V variant and other rare mutations in MTHFR in some individuals?

Fritz Roth: If a new variant comes up, a rare variant, 30% of the time it's gonna land side by side with this common variant. And so even if that common variant sort of isn't so bad and you can fix it by having enough folate in your diet, what's the impact on that second variant? If it lands in this common context?

Kortni: Dr. Roth is explaining the genetic context, or in other words, he is examining how multiple variants can have combined effects. But there is also the environmental context which makes the situation even more complicated.

Evelina: So this is the nature vs nurture dilemma?

Kortni: Yeah, but I thought genetics was complicated enough already!

Evelina: The nurture part of this dilemma is represented by the environmental context. These are your surrounding conditions, and they provide a combination of factors that can contribute to your disease risk. These can vary a *lot* from person to person. For example, your age, nutrition, exercise regimen, and stress can all be associated with your disease risk. And we're PhD students, we know a lot about stress!

Kortni: So, if genetic context and environmental factors contribute to your disease risk, could it also affect how you respond to medications that then treat these diseases?

Evelina: Great question! Yes, the field of pharmacogenomics (or PGx) focuses on this topic. That is why it's important to map the effect of variants in different genetic contexts. There are many brilliant scientists moving this field forward right now. We talked to the directors of the Pharmacogenetics Program and the Clinical Laboratory Genome Diagnostics Lab in the Hospital of Sick Children in Toronto.

Iris Cohn: Hi, my name is Iris Cohn. I'm the director of the Pharmacogenetics Program here at SickKids. I'm, by training, a pharmacist and I got into pharmacogenetics through the medication safety perspective.

Michelle Axford: I'm Michelle Axford, I'm one of the clinical laboratory directors in the genome Diagnostics lab, which is the clinical genetics lab here at SickKids, I have a PhD in human genetic diseases.

Kortni: It's great to meet you both!

Evelina: Iris, can you tell us how you began your research in Pharmacogenetics?

Iris Cohn: So when I worked as a clinical pharmacist, I always was interested in making sure that medication errors do not happen, that we continue to safely prescribe medication or dispense medications. And this was one of my side research opportunities. When we moved to Canada, 12 years ago, I had an opportunity to work at a small project called Pharmacogenetics at Sickkids. And what we did is we just looked at a MassARRAY platform which was given to us and to see if it was even useful for us, and this is how I got into pharmacogenetics. This field is rapidly evolving and is changing the way we look at medication safety.

Evelina: How about you Michelle?

Michelle Axford: Sure, I went into industry for a while as a research scientist and I came back to Sickkids. I've been certified by the Canadian College of Medical Geneticists, hence my current position. So my job is mostly related to the Mendelian rare disease space. But a few years ago, an opportunity came up for me to have a role in a smaller pharmacogenetics project within the clinical genetics lab at Sick Kids. I was fortunate enough to work with Iris on that one. But now I think, you know, in the greater context of precision child health here at SickKids, we have a newer goal within the institution of precision child health, and so Iris and I are now involved in a much larger PGx project. And for me it was a pivot, it's not really in the wheelhouse of my educational background. But I really love learning about new things and so this was a very good opportunity for me to get involved in pharmacogenomics.

Evelina: Then, the next question is, what is the difference between pharmacogenetics and pharmacogenomics?

Iris Cohn: Pharmacogenetics is the field of how genetic variation influences medication therapy. And the term pharmacogenetics and pharmacogenomics are interchangeable yet they were coined at different times. So, pharmacogenomics is really looking at the whole genome, at multiple pathways in order to find an interaction between a set of genes and the medication. Whereas pharmacogenetics historically looks at one gene which may influence the response to a certain medication.

Kortni: So how is pharmacogenetics different from genetics?

Evelina: And why is it important to annotate variants and their effects on drug metabolism?

Iris Cohn: Pharmacogenetics is a little bit more complicated than just genetics where you look just at one variant or a couple of variants which can influence disease state. Pharmacogenetics, we're talking here about the metabolism genes which are highly polymorphic, meaning they're coming in multiple variations, and many many variations. So when you then look at defining a gene, you have to annotate them because you need to understand what they are doing. And when it comes to metabolism, or breakdown, or activation of medications, and some variants are already defined, whereas others have no definition, are not defined yet, so variants of unknown significance. So therefore it's very important to make sure that we understand each variant in the gene and how it influences medication therapy. And also the most important to understand that whatever we are doing is translated into clinical practice.

Iris Cohn: And when we use pharmacogenetics in order to guide medication prescribing or efficacy, then we have to make sure that we understand the variant which lead to that decision making.

Evelina: We already learned about *MTHFR* in the context of diet and other genetic backgrounds. But regarding drug response, are there other genes important for metabolism?

Iris Cohn: There are some genes which are involved in disease state, also pharmacogenes. One of them are, called *RYR1* and *CACNA1S* which have something to do with malignant hypothermia. And they are now reported in the 75 ACMG genes.

Michelle Axford: It's 81 or -2 now.

Iris Cohn: Yeah, where do these genes which also have an implication on disease state belong to, do they belong to pharmacogenetics only or do they belong to the group of variants which have an implication in disease? The same is for *Factor V* Leiden. We have the same discussion here. It has an influence on therapy when you get estrogens. So, *Factor V* Leiden is a hereditary disease which influences coagulation, like blood clotting. And when you have that variant or you're homozygous for that variant, you have an increased risk of developing blood clots but this also has an implication of when someone starts estrogen therapy and that you're increasing the risk of developing blood clots. So these are one of those few genes, and there will be more coming in the future, where we really have to start looking at pharmacogenetics in a multidisciplinary approach.

Evelina: And for this multidisciplinary approach to become reality, we need actionable variants. So, we need to understand the effect of different variants, so that we can prevent disease and effectively screen patients and their relatives.

Kortni: But we learned in previous episodes that most variants are “variants of uncertain significance”. So what does this mean for when pharmacists have to figure out what dose of medications we should take?

Iris Cohn: We're coming here to the limitations, where when you have a gene like *RYR1* or *CACNA1S*, there are certain variants which we don't even know if they really have a significant impact for malignant hypothermia and again, variants which we don't understand yet. But that is the limitation of all genome sequencing or genetic information currently.

Michelle Axford: Lots of VUSes, [Iris laughs] as this podcast alludes to, I suppose.

Evelina: That being said, how many actionable genes or variants are currently being used at Sickkids?

Iris Cohn: So here at SickKids we're testing for around 10 actionable genes, variants in those 10 genes. And outside of Sickkids, there are another 10 genes which can be used, which have clinical guidelines available. Then you can dose adjust or prevent taking a medication, so the whole idea is really precision medicine, knowing ahead of time if a patient responds to a certain

medication and how they respond to a certain medication. The metabolism genes, the *CYP* genes, the cytochrome p450 genes, this is the low-hanging fruit even though it's not easy to identify correctly.

Evelina: The cytochrome p450 gene codes for enzymes in the liver that break down many compounds. These enzymes often break down the medications that you take, activating them so they can be distributed in the body and have a therapeutic effect. That's why there are some medications you can't take when drinking grapefruit juice, because grapefruit directly affects these enzymes!

Kortni: Oh yeah, I heard the same thing happens with Fava beans! It makes sense that our genes can dictate what drugs we can take, since they also dictate how we process the food we eat!

Evelina: So is that all there is to pharmacogenetics?

Iris Cohn: The not low-hanging fruit is now pharmacokinetics versus pharmacodynamics. Pharmacokinetics is the absorption, distribution, metabolism and elimination of a medication. Whereas the pharmacodynamics is the interaction of the medication within the bodily functions, receptors, neurotransmitters, immune system and many, many more pathways. And here we are not as good as understanding those differences and what it means to our medication response. We're not there yet. Not completely.

Evelina: And is that why contextual maps are essential at this point?

Michelle Axford: In theory, contextual maps are a useful tool, right? And outside of cancer-based testing in terms of the contextual variant effect maps, a long time ago, a group looked at every single possible missense variant in *TP53* for example, just to see what it would do functionally. And PGx is complicated, like we've kind of alluded to, changing variants in a single gene might not give you the full impact of what's happening.

Kortni: TP53, or tumour protein 53, is essential in preventing cancer. This protein can activate DNA repair, stop the cell cycle, and even initiate cell death if DNA repair fails. It's a tumour suppressor that keeps the cells from growing and dividing uncontrollably. It is considered to be the "guardian of the genome". Many scientists have looked at the effect of mutations in this protein, and researching these effects in different genetic contexts is incredibly important in cancer treatments.

Michelle Axford: You have to think about, you know, which variants are we changing in concert with each other? And how do we develop contextual variant effect maps if you have to change variants in multiple genes at the same time and, and have an almost infinite number of options to do that. And so, while I think a lot of things are very interesting in theory and I'm not saying it's not gonna work, because it might, it's gonna be difficult.

Kortni: But how do we get there in terms of overcoming these limitations? Fritz Roth has some ideas about this.

Fritz Roth: So for many genes, in fact, you just have to look at the literature and look for other people's assays that they've used for testing individual variants, and some of them are scalable to make a MAVE. And so in this case, Warren Kruger's lab had developed a yeast based assay. And the idea is basically you take the cousin gene, the ortholog of *MTHFR* in yeast, which is, you know, despite the fact that it's a billion-year diverged eukaryote has a lot in common with human cells. And in *some* cases, you can take a gene from humans and replace the corresponding gene in yeast and fix the defect that's caused when you delete the yeast genes.

Kortni: Right, that's called a yeast complementation assay, I do these in Alex's lab all the time! This assay allows us to test human gene variants directly in yeast. What is the complementation system you're using?

Fritz Roth: So, in this case, the cousin gene is *MET13*. And if you delete it, the yeast can grow on rich media, but they die if you grow them on media that is missing methionine. And, so that's basically a life or death assay. And you put in the human *MTHFR* gene and the wild-type version of that gene, the reference allele rescues, as does A222V by the way, but more severe, damaging alleles variants in *MTHFR* will fail to rescue and the yeast cell dies.

Kortni: So then how do you use this idea for multiplexed assays of variant effects?

Fritz Roth: It's like a little lab evolution experiment. Each yeast cell gets at random a different variant and the variants are introduced into this yeast strain that's addicted to *MTHFR*'s function when you shift to "minus met" media and then you wait and you see which cells dropped out over time and which didn't. And of course, the trick is how do you observe hundreds of thousands of different strains of yeast all growing in the same test tube? How does one observe to see which variants fell out over time? And that's where the awesome power of next generation sequencing comes in.

Kortni: And didn't your group also make multiple maps in different contexts?

Fritz Roth: Right! So we actually did eight maps. So forget A222V for a moment, we did four maps where we changed the folate levels, and then we did it all again where every clone, every yeast strain carrying a human *MTHFR* copy, not only had a random variant that we put into the clone, but it also had every clone, those experiments had the A222V variant. So we're now able to observe, what happens when you landed in the A222V background, and what happens if you didn't?

Kortni: So, what did you find?

Fritz Roth: If you dive in, what you'll see is that in the catalytic and linker domains that every variant is a little worse in the A222V background.

Kortni: So that means that the change from alanine to valine in *MTHFR* makes the effect of harmful mutations even worse! This mutation is a sensitizer! So, in order for scientists to interpret the pathogenicity of variants, they need to take into account all the other potential

variants that either sensitize or desensitize them. That is, the variants are *context dependent*. Are there other genes you are working on that are context dependent?

Fritz Roth: Yeah, so actually, I'd say about a third of the genes that we've either worked on or are working on have some kind of context dependent effect. So I can give a couple of hints about that. So, we published a variant effect map for the gene *HMBS*, which is important for heme biosynthesis. And there's a disease called acute intermittent porphyria, which is one of the hepatic porphyrias. And there's another gene in the same pathway called *CPOX*. And this one is interesting because there has been a pathogenic variant reported that is dependent on heavy metal toxicity. It's benign if you're not exposed to heavy metals, but it's pathogenic if you are. And so we have done a map of this, plus or minus mercury, and we definitely see differences between the two that make us think that we're able to model that. We've just recently done a map for the LDL receptor. So maybe you've heard of good cholesterol and bad cholesterol. The latter being LDL cholesterol. We found that VLDL is the sort of a bigger particle like LDL, can competitively inhibit uptake of LDL via the LDL receptor. And so we've done a map with high VLDL and no added VLDL to see which variants are important for preventing competition by VLDL. And this has turned out to be kind of an interesting story.

Kortni: Amazing research, so do you think MAVEs are becoming more common in the context of different drug conditions or pharmacogenes?

Fritz Roth: So for pharmacogenes, I guess, we haven't done a map for a pharmacogene yet, but there's some great coming from our friends in Seattle on Cytochrome P450's from Maitreya Dunham's group and Doug Fowler's group and others. And so that's an exciting development that not only could we do pharmacogenetic testing for common variants, but in fact, say something to people who have rarer variants. And there are other pharmacogenes, we're working on one called *DPYD* which is a common source of either resistance or sensitivity to 5FU which is a chemotherapy and other agents in its class. So it's, actually common practice now to do genetic testing ahead of chemotherapy using 5FU to see whether people are going to be more resistant or sensitive based on their variation in *DPYD*. But typically that testing looks at common variants only. And then the patients who have a rarer variant in *DPYD* are sort of treated as though they didn't have a variant at all. And sometimes they could get treated too little or too much.

Evelina: So Fritz touched on contextual mapping of interesting genes such as *CPOX* and heavy metals, and the *LDL* receptor and cholesterol. In the realm of pharmacogenetics the most famous drug metabolism genes are the Cytochrome P450 family, as we already saw earlier. But Fritz also mentioned the oncology-important gene *DPYD* in the context of chemotherapy response to the drug 5FU. Iris had something to say about the field of oncology pharmacogenetics.

Iris Cohn: I just want to emphasize that pharmacogenetics in oncology is a little bit different than pharmacogenetics which we are doing. When you look into pharmacogenetics guidelines which are available or genes which are looked at, you find a lot of genes in oncology for patients who have a certain tumor or a certain cancer, which is associated with specific genetic changes. In our field what Michelle and I are doing, we currently don't work in that field, it is a different kind of pharmacogenetics. One is germ line and the other one is not germline, somatic and tumor specific markers, which is what pushed pharmacogenetics forward, which pushed

pharmacogenetics into where it is now, it came from oncology. That is a completely different mapping and completely different specialty, and we are currently not dealing with that.

Evelina: Why is it so difficult to implement PGx testing when the field is advancing so much?

Michelle Axford: I think a big part of that is the technology you choose to use to do the testing. And so our lab has chosen to do this by, you know, genome wide sequencing. But the choice of technology is always a challenge. The more comprehensive the test is like genome-wide sequencing, it's typically more costly. And you have a longer turnaround time and sometimes, you know, just like for rare germline disease, turnaround time does matter. And I can imagine how in PGx that could matter very much for a child who's maybe medically complex and on a lot of medications or the clinical team is thinking about putting them on some medications. And so while targeted tests are really great and maybe have a quicker turnaround time that you have to think about the utility of them in the future when you understand, you know, maybe more variants can be added. But then you have to do a whole, especially in a clinical lab, a whole validation study that takes a good long while and, and takes up lab personnel and so, you know, the forethought to use, let's say, genome sequencing to do this is great. But then you also have to, you know, keep in mind, what will you see when you're doing genome wide sequencing? Do you want to see all the variants regardless of whether, you know their effects, whether the variant is of uncertain significance? And so it's just, it's just the thoughtfulness you have to put into the technology and how you're going to visualize the downstream results of that technology and how easy it is to scale, I think.

Evelina: Michelle, you had a great anecdote about explaining your job to laymen?

Michelle Axford: So my field of work is more the rare genetic diseases. I was on jury duty once, and when you actually get to the point where you're called in front of a judge, you have to have written down what your profession is. And so at the time, where I was working, my actual title was Laboratory Geneticist. And so I wrote that down, but when it was read out in front of the court, the person said genealogist, which as we know is someone who studies essentially family trees. And so I think even just the concept of genetics itself to the layperson is not something that people really think about all that much, right?

Evelina: This is just like being a PhD student, people think I will be a doctor that can do surgeries, but obviously this isn't the case at all! So, Iris, how can we educate people about pharmacogenetics?

Iris Cohn: So we have to be very clear when we move pharmacogenetics forward, that education is a big piece of pushing pharmacogenetics into clinical care. The understanding of the limitations of our current pharmacogenetic testing is important because people expect so much more from pharmacogenetics than we can currently offer and currently it's out of pocket, the Ministry of Health doesn't pay for it. So these are our limitations. Why pharmacogenetics is not standard of care, education, lab work, understanding of the clinical implication of genetic variants.

Evelina: Can you expand on the clinical implication of genetic variants? Are there other challenges for translating functional evidence to clinical guidelines?

Iris Cohn: When the medication comes to the market, it has to go through different phases, right? And this is very well controlled by the health authorities like FDA or Health Canada. And when the companies do the research about a certain medication which they want to promote and bring into the market, pharmacogenetics is just a side bar of it. So there is not a lot of research happening based on pharmacogenetics only. So the way how the trials are created means that pharmacogenetics is not at the forefront. And there's not a lot of money put in to try to figure out the differences between different patient populations when it comes to pharmacogenetics. There was a paper discussing that there will be an international Pharma company coming together, saying we have a problem here with pharmacogenetics research because not only do we have differences between pharma companies, but we all have differences of the regulations of genetic information in every single country. And so when you want to do pharmacogenetics research, which is actually belonging to genomics research, you have a problem that there are no real guidelines, which helps create exactly what you want; pharmacogenetics research where we look only about genetic variants and medication pre-emptively and not retrospectively. So it's a huge problem. So when it comes into CPIC guidelines, CPIC guidelines, what they do is a peer reviewed voluntary group of international researchers and pharmacists and physicians who come together and review publications about drug-gene interactions. And then they come with a level of evidence, how much level of evidence is available for certain drug-gene interactions. And this is how CPIC are developed. So there is no basic research happening the drug-gene interactions on a population level.

Iris Cohn: My research, where I'm putting emphasis is really to translate research into clinical practice. This is where I am, and to look which guidelines, which pharmacogenetics guidelines are actually helpful in the population we serve, which is children and not every guideline which is out there actually makes sense for our children, but there are guidelines or there are pharmacogenes that should be tested for certain patient population, we are adamant that they should be done because we improve medication safety and we're improving also a better outcome for those patients. So currently, I would not suggest that everyone should do pharmacogenetic testing. It should be really limited to certain patient populations. And currently, we should put the money into making a difference in the patient outcome. This is what my research is, really looking at certain patient populations. What can we do to improve to improve their outcome? Can we guide medications through pharmacogenetics? And is this even helpful?

Kortni: Michelle and Iris covered many limitations of pharmacogenetics, and so much research needs to be done to implement pharmacogenetics at scale. But what could be the future of contextual variant effects if this happens? We asked Fritz.

Fritz Roth: In a future world where everybody is sequenced at birth and I think I'll put myself out there and say it's not a question of if, but when that happens, where there's a health system that can manage this which we don't have yet anywhere in the world, I would say. But in the future, where everybody is sequenced at birth, you might be able to tell someone: "Hey, you have this common variant A222V in one of your copies of *MTHFR*. But in the other one, we

have this very rare variant that nobody's ever seen in a human before, but we've measured the function of that variant and it's about as damaging as the A222V variant. And so, you might want to get a high folate diet, especially if you're pregnant.”

Kortni: So these MAVEs are important to pave the future of pharmacogenomics at scale, but is it harder to do a contextual MAVE than a regular variant effect map?

Fritz Roth: Yes it's easier to make context dependent maps than it is to start over with a new gene. But once you start thinking about how many contexts you'd really need to do, I think you realize that what we've done is a toy project and we should use this as a tool as a jumping off point to discover more efficient ways to do things like this in the future.

Kortni: And how can we make contextual MAVEs more efficiently at scale?

Fritz Roth: The sky is the limit on what we could do given enough money, we just can't do it all. We don't have capacity to do everything, but we could model a fair amount. I think it becomes more complicated to model environments that depend on multicellular context. So MAVEs to date have been in single celled organisms, model organisms or in human cell culture. A major challenge for the field is multicellular models for doing MAVEs so that you could model the environmental impacts that are really depending on that multicellular context. So I think we can't do those right now and maybe this is a subclass of that, but pathogens are of course a critical type of environment. And that's not always going to be easy to model in a single celled organism setting.

Kortni: Wow, its incredibly complicated to design a test, validate the results, consider pathogens, and scale it to all the mutations.

Fritz Roth: I think the challenge of scale where you have to pick and choose among the environments we could model is we're going to have to find some hybrid computational experimental solution for that. So it will not be practical or desirable to test every variant by every context, because there's just too many contexts. So we're going to have to come up with strategies for sub-sampling, for choosing which subset of variants to test to learn how the variation depends on context so that we can teach the computers to predict that for us.

Kortni: Then, how do we map variant effects in different populational genetic backgrounds? It is a well-known fact that many genetic studies prioritize European and North American populations.

Fritz Roth: So it may be worth testing making a variant effect map in every common genetic context. So if there are variants that are common in a gene and I think to be equitable about that, you'd want to consider the world's global populations and consider variants that are common even in subpopulations in the world. So, you know, it may be that there's only one major common variant in *MTHFR*, but if we look at in subpopulations across the world, there will be others that are worth considering. Let's say that there are tens of common variants that are common enough in subpopulations to warrant a context specific map, then we might want to take some notion of reference background allele across many environments and figure out which ones

are the key environments and you probably want to do that on a subset of variants, not on a whole variant effect map, but having figured out the key environments and take those across the common genetic context, that seems like it should become part of the definition of mapping a gene.

Kortni: Is there a magic number of contextual maps that may be enough for precision medicine, especially for genes with multiple functions and phenotypes?

Fritz Roth: For genes where you have enough annotated clinical variation and that is actually a disappointingly small number of genes actually where there's enough variation that is really well annotated as "this is pathogenic and this is benign". But where you have that information, you can just say once we can cleanly distinguish a large number of pathogenic variants from a large number of benign variants, then maybe we can declare victory and stop. But now keep in mind that there's kind of a problem with the definition of pathogenic and benign. That pathogenic means "can cause disease in somebody, in some context". So if you're satisfied with just knowing that a variant can cause disease in somebody in some context, then you could declare victory and stop doing maps. But if you wanted to go further to an individualized notion of pathogenic and say, what is *my* risk of getting a disease, given this variant and given my diet and lifestyle and exposure to pathogens over time, then maybe you do still need those context-dependent maps even if you were able to tell from that very first map, what's a pathogenic and what's a benign variant. But now tell me who's going to get the disease, and we're not there in the field of clinical genetics, outside of a few special cases of individualized risk assessment.

Kortni: Hopefully we will be there soon! Thank you for your insights, Dr. Roth!

Alex Nguyen Ba: In this episode we learned what contextual variant effect mapping is, what is pharmacogenomics and how functional evidence is translated from the lab to clinical guidelines. We learned that producing this large-scale contextual evidence is a huge challenge, especially if we consider different populations and their common alleles, their diet, their exposure to pathogens, and other disease and medications. People in the field of variant effect mapping and pharmacogenomic testing are driving this new form of doing precision medicine; they want to consider each person individually, not only based on genetics, but in the full aspect of a being. As always, we end this episode with a series of fun rapid-fire questions, so stay tuned and listen to their answers!

Moez Dawood: Alright, so basically, I'm going to ask you a really brief question, and you have like two seconds to respond.

Fritz Roth: Oh dear, okay.

Moez: Alright, DNA or RNA?

Fritz Roth: Yes

Moez: R or Python?

Fritz Roth: Yes

Moez: Ok, you've got to actually answer [both Fritz and Moez laugh] let's start this again?

Moez: DNA or RNA?

Fritz Roth: Yes.

Moez: Fritz you've got to respond [both laugh]

Fritz Roth: No I just did!

Moez: Okay, R or Python?

Fritz Roth: Yes.

Moez: Short-read or Long-read?

Fritz Roth: Absolutely.

Moez: Single-cell or Bulk?

Fritz Roth: Bulk.

Moez: Knock-in or Knock-out?

Fritz Roth: [Long pause] Wow, okay that's a stumper, I got no answer.

Moez: Golden Gate or Gibson?

Fritz Roth: Gibson.

Moez: Coding or Noncoding?

Fritz Roth: Coding now, noncoding later.

Moez: In vitro or in vivo?

Fritz Roth: Yes

Moez: Favorite model organism?

Fritz Roth: Yeast.

Moez: Favorite scientist?

Fritz Roth: Decline to state.

Moez: Favorite media?

Fritz Roth: [Laughs] I'm going to pass.

Moez: Favorite sequencing technology?

Fritz Roth: Illumina.

Moez: Favorite piece of lab equipment?

Fritz Roth: Wow a stumper, I have no quick answer to that one. Favourite piece of lab equipment...I'm going to go with sequencer!

Moez: Favorite science movie/media?

Fritz Roth: I got nothing.

Moez: Something outside the lab that you wish you could multiplex?

Fritz Roth: Parenting.

Moez: Nice, what is the stupidest thing you've ever done in the lab?

Fritz Roth: Well, I would say that a stupid thing I've done in the lab, back in the day is pour agarose gels using water instead of buffer, but that wasn't the stupidest thing. The stupidest thing is doing that for the fourth time.

Moez: What is the smartest thing you've ever done in the lab?

Fritz Roth: Switch to computational biology.

Moez: Alright.

Adrine de Souza: Okay, DNA or RNA?

Michelle Axford: DNA!

Iris Cohn: RNA!

Michelle Axford: [both laugh] Love it!

Adrine: Pharmacokinetics or pharmacodynamics?

Michelle Axford: Dynamics

Iris Cohn: Currently the group is on kinetics.

Adrine: Short-read or long-read?

Iris Cohn: Long-read!

Michelle Axford: I'm going to say short-read only because that's currently what almost what we do entirely in our clinical genetics lab.

Iris Cohn: Everything you're telling is the future coming! Right now it's kinetics but dynamics will be coming, if it's short-read, long-read will be coming.

Adrine: You choose if you're focusing on the future or in your present!

Adrine: Single-cell or bulk?

Michelle Axford: Single-cell.

Iris Cohn: Single-cell.

Adrine: Knock-in or Knock-out?

Michelle Axford: I've always been knock-out, I just like knock-out models.

Iris Cohn: Knock-out!

Adrine: Coding or noncoding?

Iris Cohn: Coding is what we know, noncoding is what we don't know so.

Adrine: You have to choose one!

Iris Cohn: Coding!

Michelle Axford: Yeah I'm going coding too.

Adrine: *In vitro* or *in vivo*?

Iris Cohn: *In vitro*.

Michelle Axford: Yeah, I'll say *in vitro* as well.

Adrine: Favorite model organism?

Michelle Axford: I worked with mice in undergrad and they were actually kind of adorable, so I'll say mice.

Iris Cohn: [laughs] I will pass on that one.

Adrine: Favorite scientist?

Michelle Axford: That one's easy for me, Rosalind Franklin.

Iris Cohn: I will go with a woman who brought CRISPR to the forefront, Jennifer Doudna.

Adrine: Favorite sequencing technology?

Iris Cohn: Genome sequencing.

Michelle Axford: Sequencing by synthesis.

Adrine: Favorite software?

Iris Cohn: Hmm, I pass there is no favorite software for me to tell you.

Michelle Axford: I'm going to say Almut, we use it a lot in the clinical lab to help us adjudicate variants and find it very useful, so I'll say Almut.

Adrine: Favorite science movie, science book, any media?

Michelle Axford: Gattaca, have you seen it?

Iris Cohn: No, I didn't.

Michelle Axford: It's great, it's a genetics-based movie basically where people can choose the entire genome essentially of their child. So, they're born nearly perfect.

Iris Cohn: Oh my goodness, okay I have to watch it then. The Imitation Game. I think The Imitation Game, it was about the ENIGMA, the decoding of the ENIGMA and this was the beginning of how we're starting using computers. So, the Imitation Game, I'm going to go with that.

Michelle Axford: I've never seen it, I should!

Iris Cohn: Oh you should, you should it's fantastic.

Adrine: Something outside the lab that you wish you could multiplex?

Michelle Axford: Oh, this isn't hard for me. If I could multiplex making dinners, I would multiplex making dinners. It is the worst part of my day. Every day, I'm on my way home texting my husband being like, "so dinner?" it's just the worst question of the day.

Iris Cohn: I have to tell you, the lunches I have to make for my son for school. I'm sorry, it's like 25 minutes in the evening. I'm taking care of those lunches to make sure that they're healthy and he likes them, so yeah, absolutely.

Michelle Axford: You know what, I'm going to change my answer to that making my son's lunch. Yeah. If I could multiplex that, it's worse than dinners actually.

Iris Cohn: It is worse than dinners! [Both laugh]

Adrine: What is this stupid thing you've ever done in research/lab?

Michelle Axford: I have a good answer for this one. Can I go first Iris?

Iris Cohn: Yes, please go ahead.

Michelle Axford: The dumbest thing I ever did which also leads into the next question which is the smartest thing I ever did, but I'll go with dumbest thing first, I was an undergrad and I was sterilizing basically pieces of glass in a fume hood using ethanol and a Bunsen burner. And I somehow set the entire fume hood on fire.

Iris Cohn: The stupidest thing I've ever did was last day and I think it was third semester pharmacy school and we had to clean our lab and I was already in vacation mode and my friend, a friend of mine, we had to take ammonium back to its place and it was a heavy, very, very big. I think, I don't know, 20 L of ammonia and I thought that it would be the smartest thing to do it without any protective wear. And I actually have a mark, a burn mark of how the ammonia really burned through my skin. A very, very deep hole, made a very deep hole. So, I always remember third semester pharmacy school.

Michelle Axford: That's terrible.

Iris Cohn: Wear your protective gear! I don't know what I was thinking, so yeah, that's the stupidest thing I did. And I didn't realize it until much, much later.

Adrine: What is the smartest thing you've ever done in a lab or in a research environment?

Iris Cohn: Honestly surrounding myself with smart people who have the same drive to push pharmacogenetics forward. That is the smartest thing I've done.

Michelle Axford: Oh, no. My answer is going to seem quite silly because it's related to my fire. The fire I set, I managed to think quickly and smothered it rather than trying to put it out with like fanning the flames or with water because I would have just spread it. So yeah, I put my lab coat on top and closed the hood and smothered it cause no more oxygen could get in, and it was put out. There was a brief moment of sheer panic as I watched the fire spread over everything that was inside the fume hood.

Adrine: Thank you so much!

Alex Nguyen Ba: Thank you Fritz, Iris, and Michelle for joining us today. And to all of you listeners, I really hope you enjoyed today's episode on pharmacogenomics, and don't forget to tune in for the next episodes on Variants and Us, where we'll discuss mitochondrial disorders, AI in variant classification and much more!

[Outro music plays]

Your genes on drugs: context matters! Episode 3 Show Notes

Further reading on the eight variant effect maps of MTHFR produced by Dr. Roth:

Weile, J.; Kishore, N.; Sun, S.; Maaieh, R.; Verby, M.; Li, R.; Fotiadou, I.; Kitaygorodsky, J.; Wu, Y.; Holenstein, A.; Bürer, C.; Blomgren, L.; Yang, S.; Nussbaum, R.; Rozen, R.; Watkins, D.; Gebbia, M.; Kozich, V.; Garton, M.; Froese, D. S.; Roth, F. P. Shifting Landscapes of Human MTHFR Missense-Variant Effects. *Am. J. Hum. Genet.* **2021**, *108* (7), 1283–1300. DOI: 10.1016/j.ajhg.2021.05.009

Further reading on the challenges of conducting pharmacogenomic studies, and the work currently being done by pharmaceutical companies to overcome them:

Bienfait, K.; Chhibber, A.; Marshall, J.-C.; Armstrong, M.; Cox, C.; Shaw, P. M.; Paulding, C. Current Challenges and Opportunities for Pharmacogenomics: Perspective of the Industry Pharmacogenomics Working Group (I-PWG). *Hum. Genet.* **2022**, *141* (6), 1165–1173. DOI: 10.1007/s00439-021-02282-3.

Profile of Dr. Michelle Axford for the Temerty Faculty of Medicine at the University of Toronto:

<https://moleculargenetics.utoronto.ca/news/medical-genomics-clinical-spotlight-get-know-dr-michelle-axford>