Introduction to Ryan Smith's Background

Ryan Smith attended Transylvania University and graduated with a degree in biochemistry. After finishing all the educational curriculum and passing Usmle step one, he decided to leave and open up a pharmacy in the United States that focused on peptide synthesis and formulations for pharmaceutical preparations.

That pharmacy tailor made compounding became the fourth fastest growing company in health care in the U. S.. Ryan exited Tailor Made in late 2020. Since then, Ryan has opened many businesses, including Tru diagnostics, a company focusing on methylation array based diagnostics for life extension and preventive health care.

Two diagnostic is a Clea certified lab and health data company focused on serving integrative and functional medicine providers in the United States to diagnostic has a commitment to research with over 21 approved clinical research studies investigating epigenetic methylation changes of a variety of longevity and health interventions.

Since two diagnostics inception, they have created one of the largest private epigenetic health databases in the world, with over 13,000 patients tested.

So it's great to have you on the show, Ryan. I'm really looking forward to talking to you about, all the really interesting work you're doing at two diagnostic.

And we're going to get into DNA methylation and relationship to telomere biology. Which are also doing some fantastic predictive work there. But you're a young guy, and, you know, you've co-founded this great company.

I first met you when you were working, in a tailor made. Tell me a little bit about your your journey from from pharmacy through to, molecular diagnostics.

Yeah, absolutely. It's been a wild one, that's for sure. I, I think that, you know, my background even before that is, you know, sort of biochemistry undergrad, a little bit of medical school.

And it really got to the clinical portion of medical school past years. Emily. Step one did pretty well, but got to the clinical stuff and just couldn't imagine doing it forever.

And so, through or through sort of, pure serendipitous. Like I, I ran into this idea of compounding pharmacy and particularly, you know, using a lot of my background to specialize in peptide and protein synthesis.

And so that's what we really did at Telerik. Compounding is sort of bring a lot of peptides to market for the first time for clinical use. And, and that introduced me to a whole new realm of medicine that, that, that I didn't know existed.

This idea of preventative, you know, optimizing and sort of functional medicine, and, you know, the medicine I had been previously, you know, exposed to was really dealing and treating sick patients.

And I always felt that, that was, you know, difficult because, you I didn't really think I was impacting change. I think that I was, you know, staving off disaster, but not positively changing medicine.

And so, I got to sort of be exposed this role for the first time via tailor made compounding and, and, and with that was really, really exciting because there's a lot of new and innovative products coming out that and as we learned more about just the baseline science, which, you know, continues to surprise me every day, the idea of this preventive medicine became, you know, even more ingrained into my mind.

And so, you know, as we considered how to vet some of these other therapies that we were doing, some of these new and innovative molecules, I was always looking for for different biomarkers, which might be able to provide a

lot of information, and make sort of these investigations a little bit more feasible.

And that's when I was introduced to this idea of epigenetic methylation and biological aging through epigenetic methylation, where you might even be able to do, you know, instantaneous, you know, 40 year placebo controlled trials through, these predictive algorithms, which then can tell you how you're changing and mitigate risk.

And that, to me was something that was incredibly exciting and something that I wanted to be involved in, particularly whenever started to become very clinically relevant in 2019, with the advent of some of these, interventional trials, like the term trial, which showed changes in biological age, which then can can, you know, theoretically show that we can reduce those risk factors as we age.

And so that's sort of the trajectory that I had taken. And now I really committed to this idea of preventive medicine and, and also this idea that age is, is one of those markers that we can hopefully treat as a preventative marker, since it has such a high correlation to all these chronic diseases.

Yeah. I mean, that's that's absolutely true. Aging is the the aging process is the bedrock of all the chronic diseases. And to be able to measure it is, sort of a holy grail within, within the field.

What DNA Methylation Measures

You need that technology to, to vet, things like the peptides and all the other therapies that are coming down the line. But for our listeners, who, you know, I'm sure most everybody has heard of DNA methylation, but maybe a little bit of a primer on, what exactly it is, what we're looking at, and how it works.

Before we get into sort of, how it's utilized and what the the really interesting stuff you're doing it through diagnostics is. Yeah. So, so the way that I, you generally like to describe DNA methylation is, is as, talking about every cell in your body has that same baseline DNA sequence, but obviously, you know, our skin cells are a lot different than, you know, the heart cells we have in our body.

And so what influences the the sort of the baseline DNA to the change and what actually is occurring and, and one of the biggest changes that is these epigenetic mechanisms, which include things like acetylation, you know, mRNAs, and then also lastly methylation, which the methylation is typically put on certain parts of our genes to silence transcription of that gene.

So to make sure that some things are turned off, and as mammals, we are have some unique, methylation, sort of signatures. So, for sort of by default, we have much more methylation in our system than other animal species.

And one of the things that happen as we age and these methylation changes can sort of change in very, very predictable fashion. So generally, as we age, more of our genome becomes hyper methylated.

And, but in particular, there are certain spots of the DNA that are highly associated with that change as we age, not just with hypermethylation, but sometimes with hypo methylation.

With some of those methylation markers get turned, taken off. And, and therefore the gene transcription is more likely to be turned on. And so so I think that this is, just from a platform standpoint, this is how our body regulates and uses all the capacities of our DNA.

And, and methylation is one of the many types of epigenetic changes which can occur to really turn off or silence that sequencing. And through some of these investigations that have been happening, now that we're getting better ways to look at methylation so much like the, you know, the Human Genome Project, whenever it first started doing methylation work was very expensive and it didn't yield a whole lot of results.

But now as the technology's been advancing, we've been able to really see a lot more from these markers. And due to the other advancements in technology such as computer learning and artificial intelligence, we've been able to now make sense of it as well.

So it's not just this abstract data, but but, correlated to certain types of health markers or even non-health related markers, even more trait markers or, or other things, which then can give us really good insight into what's happening with an individual.

And, and then how we might be able to mitigate change. And so this is a sort of a new field. It's evolving due to breakthroughs in the, the actual benchtop testing as well as the interpretation of, of this through computer learning.

And so a lot of really exciting things as we sort of have this whole new book that we get to interpret and read, and we're trying to really create that Rosetta Stone to understand exactly how to read it and what that information means.

Yeah. I mean, when I was first introduced to the field and thinking about it, you know, and certainly back when the genome was, first, you know, decoded, we thought that that was going to give us all the information, but, you know, that there's absolutely good information from that.

But but really, the changes in gene expression, you know, what, Michael Fossil likes to say is, you know, what makes a nose a different from a toe, is is sort of a more important kind of thing that that we're learning as a biomarker of aging, though.

Another thing that, I think maybe we've talked about in the past, in some of our conversations, is predicting chronological age is sort of important. But, for forensics.

And when the first biomarkers came out with, Horvath, predicting very highly a r squared off like.96 or something, and then the Weidner clock where you only need three CPGs to have, you know, very high predictive, but predicting chronological age, unless you're in forensics where you want to know, you know, what the ages of a piece of tissue from a dead body is not that important.

Because if it's really good, then you just use chronological age. So one of the changes I think that's made me more interested in is now now these data sets, these clocks are being trained on different kinds of data sets, not trying to predict chronological age.

And that's, I think, kind of some of the interesting stuff that you've been doing. And I think what makes them more interesting clocks. Would you tell me a little about what what kinds of training, is being done and what you're looking at?

Yeah, absolutely. You know, I think that that, that is, you know, well put the idea of knowing your chronological age, you can really just ask. Right.

And so in again, chronological age is still highly correlated to aging. So not completely useless. But with that being said, and I should also mention, changes in predicting this chronological age also then might intuitively tell us things that change some of the phenotypes of aging as well.

And so, so, you know, I, it was really exciting for its development and sort of showing this high link. But but the new developments are definitely about all training these markers, to predict outcomes or phenotypes.

And so, the really, that sort of separates what they call the first generation versus the second generation clocks, where the first generation were trained to predict chronological age, whereas the second were trained to predict some other type of outcome.

The two best examples are Morgan Levine's, You know, age, which used really ten blood based markers to create this idea of a phenotype of, sort of, of health.

Second-Generation Epigenetic Clocks

And then, secondarily that image by Doctor Horvath, which is trained against time till death to be able to predict,

sort of lifespan and so those are really, really exciting and, and really make this a lot more medically relevant, because if we want to stave off the, the, the, you know, those predictions, then we can look at ways to change those, and give us a little bit more of a health outcome.

Yeah, that's that's a great explanation of it. The those two clocks are sort of the most important second generation clocks. Maybe also for our audience, a little bit more detail what you mean by when something is trained on the data.

I use that phrase. You use the phrase. But maybe, our listeners don't know exactly what that means. I when I first started reading about it, was it was a little complicated as well.

Definitely. You know, I'm not sure that I even have the best words to describe it, but when I say trained, I generally been looking for correlations, right?

With computer learning systems. So the idea that, you know, we're gathering large and robust amounts of data. So even with, you know, some of the first or second generation clocks, they were using 450,000 or 850,000 locations on the DNA.

Each of those location gives you a number, essentially a percentage of methylation. However, whenever you come up with the final algorithm, which is used to predict those outcomes, you might use something like 500 or 1000, so you're significantly reducing that to the things which matter most, which have the most predictive capability.

And so, so we sort of training the, mathematical algorithm then to predict that outcome, which you're sort of correlating it against. And so, so that is sort of what it's meant by training.

And, and now though, this is taking on sort of a life of its own. It's as the aging concept has proven that there are some clear methylation signals which then can link to other outcomes.

So some of them, one of my favorites actually, even, probably something very topical at this point is just a few days ago, a week ago, there was an article published, with methylation algorithm that could predict schizophrenia in over 80% of individuals.

And so, that was really exciting, especially with some of these mental diseases where, you know, we're looking at blood methylation. That's another really important, I would say, concept as well, because, every cell has a different epigenetic signature, as I mentioned, right.

The skin cells and heart cells are going to behave and have different epigenetic expression. So, whenever you train these things, you have to train it on a cell type specific, or at least use the same cell type investigation method to create the algorithm as you're going to use it in clinical practice.

And so, for us to be able to see, you know, diagnosis, it's gets a pretty, you know, with high levels of accuracy and blood based methylation. It sort of comes up with this idea that we can really train every outcome, that we really wanted to look at against these methylation marks.

We might have different degrees of specificity and sophistication. However, the idea is that this as a platform has so, many robust markers and the computer learning is getting, you know, so, I would say well trained that you can really predict multiple different outcomes.

I think aging is definitely one that is probably the most exciting because instead of just a correlation, it looks to be maybe a causation at this point.

And so that that's exciting. I think from a mechanism of action, we still don't know enough to sort of tell you what that of is or that causal mechanism.

But but I think that that's why aging is probably the most exciting. But this epigenetic platform in general can do

everything from, you know, predicting, athletic performance to telling you your immune cell subsets to telling you, you know, your list of exposures that you've had across a lifetime from certain chemicals or, or diet and nutrition or etc..

So this will be a platform that continues to evolve, but aging looks to definitely have a special place in this idea of epigenetic methylation. Yeah. So the, that's great to be able to I mean, I was reminded of sort of the same issue in, in telomere length measurement, when you're talking about which cell type that you're measuring it in.

So, you know, we know that every cell has telomeres and they get shorter with age. But what we're measuring in a blood test is, you know, the killer length of the white blood cells, depending on which, as you use it could be, you know, pbmcs or lymphocytes are great insights, but there is pretty good predictive.

You know, algorithms and algorithms in prediction of outcomes in chronic diseases, cardiovascular disease, Alzheimer's disease, osteoporosis, etc. from, from telomere length, even though it's not the cells of those tissues.

So the same thing, I guess you're saying you can do with DNA methylation age. Now, I know that Horvath has looked at the DNA methylation signatures in individual tissues, and he has a pan tissue clock and he has the individual clocks.

But there's pretty, pretty good correlation. And so what you're saying with the schizophrenia is that it's fascinating that we can look at blood and see what's happening in the brain in terms of, complex mental illness.

You know, that is pretty fascinating. But in terms of the, the sort of the, degree of, of accuracy, the clocks that predicted chronological age had, you know, an R squared, you know, which is in a, you know, 0.9 range, which is amazing.

These other clocks for predicting things like lymphocyte subsets and other things. They're low. Right? I mean, what's the range for those. Yeah. Definitely lower. You know, it all depends on the algorithm.

You know, in terms of which one you're using and looking at, those are squared. But, but there are definitely lower. And they're getting, you know, much, much more improved as the time goes on.

You know, as I mentioned earlier, that schizophrenia predictor has a very, very high correlation value. Whereas, you know, some of those immune cell subsets have wide ranges of error, depending on on how you're using it and what data set you're looking at.

And so those are getting better, you know, and the benefits of those particularly for immune cell subsets is that, you know, one of the things that is really helpful in all of these investigations is you need to train it to a phenotype.

And oftentimes that means outcome phenotypes. And so, that data can be hard to get right if you need to really have, patient samples from, you know, 20 years before they develop a phenotype.

And that data is really hard to get, especially as we talk about things like immune cell subsets, where you might have to store pbmcs in high volumes and stability can be difficult, and you have to collect it in the right way and then get into storage within a few hours.

And so some of those things like, you know, immune cell subsets, I've been, you know, really exciting because our immune system changes according to a lot of different diseases.

But now, by having a surrogate marker that just looks at DNA, we can we can then apply this to a large, lot larger data sets from Biobanks that go back 40 or 50 years.

And so the idea is that that, there's it might not be as accurate as traditional immune cell subset testing at this time, but it's still exciting because we can do it in retrospect, because DNA is a little bit easier to store.

There's no stability concerns, there's no types of draw concerns. And then we're able to look at some of those data to look at how the immune cell subset is affecting, you know, disease prevention and risk.

And so that's exciting. Even though they're not as accurate at the moment, they can still be, you know, sort of helpful clinically. I, I always use some good examples from some of our practitioners where we do some of their immune cell subset testing.

How Methylation Data Is Trained

And just to clarify on what that is, we're sort of telling you the percentage of your different types of immune cells. And we're not telling you an absolute concentration, just telling you a relative percentage.

And so one of the things I want to do there, so I just think that that's an interesting point. So you, you can't give, an absolute count, which, you know, you would think would be difficult.

You can give a percentage. So you can give, the ratio of Cd8+ to CD4 as, you know, helpers, suppressors to helpers. You can you do it for natural killer cells and, and B lymphocytes and things like that.

And I'm just curious what kinds of things are we looking at. Is this because those cells have, certain, gene transcription that then you look at the methylation patterns of those.

Yeah, absolutely. So the idea is you in order to create these methods, deconvolution is a very, very exciting thing, not just for, you know, lymphocyte blood based tissue, but for any tissue. Right.

If you were to even look at a cancer cell, for instance, and look at, you know, regions that might have this genetic, predisposition or polymorphism versus others.

And so, so this whole field of deconvolution really at first establishes a reference, where you look at an individual cell type. So we might look at natural killer cells, and then look and compare that to your eosinophilia.

And we might say between these two investigations, what is different, you know, and what is different to a high degree. And so whenever we define what is different, those are called differentially methylated regions.

And the good news is for a lot of these immune cell subsets, these differential differentially methylated regions are robust enough to be able to tell, you know, how much we're finding of this region versus of this region, and then being able to combine that to get an idea of those percentages, and again, more developments are being made to break those down into more advanced subsets.

You know, senescent cells is one that is, you know, we've talked about before and is very exciting, to be able to finally quantify, senescent cells in a way that is a little bit more reliable than, than some of the other methods at this point.

And, and a little bit, you know, less expensive and easier to perform. And so that's exciting. And, so we're looking at those different methods, regions.

And that's really honestly what we're doing for, for most investigations is we're defining the areas for a certain type of phenotype or a certain identity that is different than what we would traditionally expect.

And by looking at such, you know, 900,000 spots, those can be, relatively robust. We might have 100 to, you know, 7000 spots which are differentiate methylated regions. We then we can break down subsets.

Instead of just knowing CD8 cells, we can know all the different types of CD8 cells or, you know, those types of things. And so, really the in order to do that, you just need those individual cell types sorted and profiled individually and then, having those same, identifications done on the larger tissue as a whole or the larger blood sample.

And so that is becoming much more robust. And there actually are ways to quantify the absolute quantity as well, using some new plasmid technology. But but we're not there yet.

And so but the idea is that hopefully it will be there here very, very soon, which is, again, hopefully another exciting development, which just goes to show you all the broad level impacts that that this is a biomarker methylation is a biomarker can have on, on sort of the health care system as a whole.

That's a great segue into, sort of a more clinical sort of look at the application of this in, in sort of day to day use. As I said, I wasn't that excited about using DNA methylation in my practice because, you know, when I started ordering it from a company that was looking mostly at predicting chronological age, I didn't see a lot of variation, which just shows how good it is as a forensic test, but then it's not that useful.

So your company is looking at it somewhat differently? In a couple of ways, which I'll let you explain in a minute. But you have a true age. Which is you can explain exactly what that is, and then you can break that down into intrinsic aging and extrinsic aging, which brings in this concept of the changes in immune subsets that can alter, you know, your DNA methylation pattern.

And then, at some point, we want to talk about the really exciting thing that you have, which is, you know, the rate of aging that you've gotten from the need in cohort in, in New Zealand.

So, yeah, why don't you, as a clinician, tell us how you would use it, why your test should be used in a practice, so we can, you know, give us some, know, take home money back.

Monday morning, you said it. Definitely. So it's it all comes down to, you know, I think that everyone who hears about this testing is sort of impressed with the correlation to age and the correlation to predicting age outcomes.

But the one biggest limitation and the thing that I think is, is even still to this point, preventing widescale adoption is the question, what do I do about it? Right.

You know, how do I change this metric? And, you know, what's the use of testing if I can't make a difference? Right. If you can't change something, then then does it really matter if you know what it is?

And, and that has been one of the biggest restrictions of this testing. And one of the reasons that we we still break this down into intrinsic versus extrinsic aging.

The idea there is that extrinsic and intrinsic aging have been looked at in many, many data sets, from the very beginning. And so we know more than most other algorithms what changes these metrics.

And so we have ideas of treatments. Unfortunately, to date there have only been five interventional studies which looked at a baseline measurement of, epigenetic age, a treatment and then an outcome.

And those studies, have various degrees of robustness as well. So, the first one as I mentioned, only had nine patients, right? The, you know, none of them have had had, you know, I should say, none of the non epidemiological ones have had, you know, over 40 patients.

And so these are still early investigations and, and and so they have some limitations to them.

Clinical Use of Intrinsic vs Extrinsic Aging

And so that's definitely something that we're trying to do is to add context, to expectations on interventions, everything from bariatric surgery to plasma exchange to stem cell therapy to even just simple things like diet and nutrition, exercise, stress management, things that are more accessible to to people without even having to have medical intervention.

And so so we're definitely trying to build that up and to be more robust. But that's the reason we break it down is

because there are different risks and there are different treatments associated with intrinsic versus extrinsic aging.

So by looking at an individual, you can sort of start to say, I might recommend these interventions versus these, and it allows you to be a lot more personalized.

You know, I think as you alluded to, the algorithm that we are I think by far the most excited about is almost what I would consider a third generation algorithm, where some where they just interrupt, I feel like is, I definitely want to that's the most exciting thing I want to get into. But, can you explain what the difference is between intrinsic and extrinsic and, you know, I know that I have patients and I myself have a significant difference between my intrinsic aging and extrinsic aging.

And you know, which one's more important or not so much. Which one? What is what is each one tell you? And I think you're right that, you know, different interventions will move the needle on each of those differently.

But, how do you go about deciding? I mean, the original clocks were sort of talking about that. Yeah. Yeah, definitely. So the the idea about intrinsic age is that, it is sort of a baseline fundamental process of aging.

And what I mean by that is it's not confounded by some of the effects we might see in the organism as a whole. That also changes with age. One of the biggest effects is probably, you know, a topic I'm sure you've just got to start with some of the other people in this series, which is immuno senescence, right?

This idea that as we age, our immune system gets worse. And, you know, just being, you know, still in a pandemic, I'm sure it's a lot. You know, another thing that people can relate to is it's the reason we unveil, these vaccine distributions in our elderly populations.

First, because they're more at risk because their immune systems are not as robust. And what that also means is that the cells in our blood, those immune cells that that constitute the majority of the things that we're testing also change in concentration.

And so, the idea is that, you know, we might have more natural killer cells, but less effective natural killer cells. We might have more, senescent cells, less naive T cells.

And if we're not accounting for that change, we can get some weird readings. And, and so, the intrinsic age sort of, uses the estimation of immune cell subset percentage to then control for any change over time.

So it is it is able to sort of factor out those immune system changes. But sometimes we don't want to do that. Sometimes we do want to get a good idea of how the immune system is changing and how that's impacting this idea of, of chronological age prediction.

Particularly in the event of, you know, death prediction or overall longevity, and also in cancer risk, which, which also should be, you know, relatively intuitive because the idea that immune system can help clear or prevent cancer is one that's, I think, widely accepted.

And the link between T cell subsets in aging, and longevity have also been well connected. So so the extrinsic age is tied probably a little bit more closely to things that relate to the immune system.

And so, so as a result, for most people who have healthy and robust immune systems that metric is going to be a little bit lower probably than it would be for the intrinsic, which we almost always find is a little bit more accelerated.

And so, so, so with that being said, it's hard to say which one is more important. I think it also depends on, yeah, as an individual, what you're trying to prevent against or what some of your other predispositions might be.

But the idea is that it gives us a better idea of the full clinical picture to make the appropriate decisions based on the the way that you want to control your own health and and address your own preventative medicine.

And so, so, so I think the more information is better, and that really helps us break it down into to understanding how the immune system is working versus this fundamental, baseline process of aging, which, you know, and sort of discussing that.

I always like to use the analogy that, you know, Doctor David Sinclair uses in his In his Lifespan book, where he talks about the information theory laws, where, you know, I whenever were first born, all of our epigenetic expressions perfect.

What should be turned on, it's turned on, which should be turned off is turned off. But as we age some of that, that regulation becomes a little bit, you know, less ideal.

And that leads to this progressive loss of information, which might also lead to the progressive loss of function, which is sort of that definition of aging, which is, again, why we think that this might be a causal mechanism for the aging process rather than just a correlated process.

That's a great explanation. I was thinking, when you said that, you we just published, study looking at the effect of T 65 on, immuno senescence, as defined by the Cd28 negative cells as the suppressor cells, CD8 positive cells that have lost the expression of this important marker, that allows them to proliferate briskly when they encounter their antigen, and that number increase is as we get older, but particularly when we have chronic viral infections and particularly CMV.

You I was thinking that, you know, it would be interesting to see, since you're able to do that kind of deconvolution, whether or not we would pick up the same signal just doing, extrinsic DNA methylation testing.

If we substituted that for the immune subset panel, and that might be, an interesting thing to do. Yeah. Very interesting. You mentioned that actually, because just even very recently there was a algorithm that can predict CMV infection.

So, and so, you know, I, I'm sure it's gonna be that paper. I will I so, you know, the I think the idea is that, that maybe you can even look at some of those different methylated regions for CMB infection compared to those immune cell subsets, the, you know, differentially methylated regions and, and maybe even have, you know, some idea if the same thing that you saw with your most recent publication is also reflected in some of those epigenetic methylation and maybe even possibly the mechanism that previously unknown for the reason to survive is having such a positive benefit.

And so we, we get we can actually even do that analysis, I think, without having to actually, get into any specific data sets, but just sort of looking at, regions and, and locations of methylation, to get a good idea if there, there might be a reason to explore further and you just need, serum for that. Right.

But what do you need? Right. Yeah. Yeah, yeah. So, yeah, you might have to talk about that. The other reason I bring that up is that a very, powerful predictor of mortality in older patients is the ratio of CD4 to C8.

Your two four and six year mortality is done in the, looked at in the, octo and donor trials in Sweden is, you know, 40, 50% increased if you are in the, what we call the immune risk phenotype below one, the CD4 to C8 versus above one.

And, you know, you're right. I mean, if you can predict those, those, those subsets, then that would be interesting thing to, to look at as well. What if they have serum from those trials just to maybe ask them about that.

That would be really. Well, so, so yeah. So there's, you know, great work that can be done with that. But, what your tests can also do, which is fascinating, is give a rate of aging.

And I was incredulous when I first heard this because I'm sort of like, you know, I'm not great at calculus, but I, I think that it's you need more than one time point to get, to get a rate of aging.

And in fact, you know, explain how you, you've done that and what benefit it has. And, and how, you know, you can have two people with the same DNA methylation age, either intrinsic or extrinsic, but have different rates of aging at a particular time point, and why that is and why that can be really useful clinically.

Yeah, absolutely. This is the one that I'm most excited about. And I was sort of referencing earlier more of, you know, almost calling it a third generation clock because it's not looking at the overall organism.

Right. It's looking at a set point in time. And that can be useful for a lot of reasons.

Rate of Aging and the Dunedin Study

One of the big reasons is that up to 40% of some of these other previous clocks, and including the second generation clocks like Grimm age and Fino age, 40% of that can be decided based on hereditary factors.

You know, epigenetics is is even passed down through our DNA, which is, both exciting and scary. I think, to some degree, you know, for instance, you know, Jews who have experienced, you know, periods of famine, that epigenetic signature can actually be even found several generations later.

And, you know, a lot of people want to be in control of their own destiny. Some people might have to work harder. And epigenetic change is still doable.

But the this idea that, hereditary factors can play a big role might give some people, a baseline context, which is not the most ideal, or, for instance, people who are trying to turn around their lifestyle from an aging perspective, but who now, you know, had some, you know, some introduction to this and why this is important.

And, you know, we're trying to lose weight and trying to eat healthy. The idea is that if we took a snapshot of their overall health, they might have already accumulated some of those epigenetic markers, which increase their overall biologic age.

But if we were to look at their instantaneous rate, what we would see is significant improvement, right? We would probably see that their, you know, their rate of aging is now decreased from what they were previously.

And that that's exciting because we're able then to vet things like interventions even with, you know, healthy people to say, you know, x how does x, y, Z, you know, affect me versus affect you.

And so, so that's really exciting in practical application, but it's also exciting in terms of how they actually created this metric. Because as you mentioned, you know, you in order to create a pace, you were an average, you know, sort of velocity.

You do need to, you know, an idea of, you know, sort of where you're coming from and where you're going. And, and so this is a really unique study that probably won't, won't be replicated.

Because it started really in 1973. And so in 1973, in a town called, you need in New Zealand, they started measuring, over a thousand patients with, with, many different phenotypic biomarkers at age three.

So things like cognitive processing speeds, everything from gum health, from dentistry exams to, you know, functional brain images to, to, you know, standing and walking and physical function measurements as well as even skin appearance.

And so they measured a lot of these different phenotypic, biomarkers, and then over the course of now, you know, sort of now that they're these people who started at age three are now over age 45, they were able to get this idea of trajectories of aging as it related to those consequences.

So instead of looking at like the second generation clocks at really large data sets, where the samples have been banked in the past, they were able to track the same patients from three years of age to 45 years of age and

quantify this idea of their trajectory to, to these, these aging phenotypes.

And then then creating an overall score of that phenotype and regressing that against DNA methylation. And what they found was sort of astounding that one of the things that I love to talk about this study on is that even things like mental processing speeds at age three were predictive of health outcomes at age 45, which goes to sort of, you know, I would say which, makes us maybe believe that aging rates are even set, in adolescence, even in infancy, our aging is, is is determined and can be mitigated and managed.

This whole idea that that early lifestyle factors can influence your rate of aging and then that influences your risk for these disease predispositions.

So maybe our concept of aging on its head a little bit where we where we sort of talk about people in their, you know, their 40s, you know, starting the aging process.

It's not I think there's evidence now to suggest it's not that way, that the aging process can even start even earlier than that. So that's exciting, too.

But the idea that you can quantify this and, and predict health outcomes, by an instantaneous rate becomes more exciting, because it is linked to so many different health phenotypes.

And also, I think as we've talked about before, oftentimes you have this juxtaposition of, the quality of life versus lifespan. And I think that this is a really interesting way to tie all of these together, because the rates of aging were actually predictive of things like, you know, sarcopenia and balance testing, grip strength, cognitive IQ and memory processing speeds and even facial appearance.

In terms of how old you look. And so all those things we mentioned, don't necessarily, you know, tell you how long you're going to live, but they, they can improve your quality of life.

And that's also exciting. So, so I don't think that they're mutually exclusive. I think that you can consider, you know, treating aging and then having improvements in both the quality of life as well as, as your overall health span.

And so I think that's exciting as well. And so that algorithm is very unique with the data set that's going to be very hard to replicate. And it's already been started to look at in some type of interventional clinical trials.

The one that is most notable is the calorie study, where they did 20% caloric restriction and showed significant decreases in the rate of aging over the course of two years through the caloric restriction, meaning that it restriction might be a good way to to improve aging rates across, many different populations.

So so, the rate of aging is looking at, I guess, different CPGs than the Act than the other clocks are looking at because it's picking up something. It's happening right now in terms of changes in gene expression, because you've stopped smoking or because you started exercising.

And that that's how it works. And you're able to do that because this is unlike the vast majority of, clocks that have been trained. This is trained on longitudinal data.

Yeah. Absolutely. And I'm sorry, I didn't I didn't answer that question. You know, first. But that's exactly right. Is it this one thing because of the addresses, these are different algorithms.

So they develop on different data sets to develop on different populations. And so they're also trained against different outcomes. And so there you would expect that there would be some overlap.

And there is there is some overlap with these individual CPGs, but there's not much. However, you still see that, for instance, those people in our data set to improve their rates of aging over time also tend to improve their overall biological age over time as well.

And so they definitely look to be at least, you know, correlative even with very few overlaps in the actual things we're measuring. And so, so generally, what we often try and say is encourage people to really look at the rate of aging.

And because it's a little bit more sensitive, as well, you know, one of the things, one of the metrics that we often look at, to determine if a test is reliable or it's a good clinical biomarker, is the interest sample variability, right.

If you test the same sample twice, you know, what is how accurate is that that reading. And and that's been you know definitely one of the biggest limitations again with epigenetic testing as well.

Even the the Doctor Horvath original 2013 algorithm had a mean absolute error of around 1.9 years, which means that unfortunately, if you were to have an investigation with that metric in a period of 1.9 years, it becomes very much more difficult to see a statistically significant result or change, and learn what is actually changing that metric.

The, the it's different with this unit because it's much more accurate from a sample variability standpoint. You know, one of the one of the interesting things is Morgan Levine from, from Yale, who created Fino age with Doctor Horvath at UCLA.

She just published, an update to all of the traditional algorithms, called these the principal component analysis algorithms, which significantly improved the reliability and decrease the mean absolute error of those algorithms, which is a huge step forward.

But it's still, even with those improvements, the ICC or sort of this measurement of interceptor variability, it's still best in that you need an algorithm.

And so, by, by sort of focusing on that rate of aging, you can maybe imply that you would then see improvements in the other overall biological ages, and it's more sensitive for more frequent investigations.

And so as a result, it is, maybe a little bit more exciting, a little bit, probably more, useful clinically than some of those other aging algorithms.

So I have had a number of patients have gotten their reports back, and I've got mine back. And when you, report out the rate of aging, one is sort of average.

It's you're aging one year for one chronological, year, and you go from point six, I think, to 1.4 when you say it's a pretty tight in terms of its variability.

Would you say that if I went from point eight to point seven, that that would be something that is meaningful? What is the actual, plus or minus on excuse?

We're talking about fractions here. Definitely. Yeah. So so it is that is a significant change. Absolutely. If you're changing by around, you know,.1., that would absolutely be significant.

It really if we see any change above really 0.05 or 0.06, that is when we consider it a significant change, which, unfortunately that might seem, you know, like, in the overall biological aging scheme, minutia.

But, but but it means it is still significant and so, it's, I think change like that is still very, very, you know, helpful. And we can say that we have seen that with some of our interventional trials.

And so, you know, from a p value calculation point that would be significant. Yes. So in the calorie trial, we were able to correlate that where you saw that, it was a two year trial.

You said so, correct. When you did rate of Aging at, how many time points.

Telomere Length Prediction from Methylation

So unfortunately, I'm not sure. So the majority of that data, was done. And I wish I could take credit for this entire project, but but we've, I've been lucky enough to license this, from a joint collaboration from Duke, Doctor Dan Belsky from Columbia and then the University of Otago, which is based in Dunedin, New Zealand.

And so, so we unfortunately have not had access to that calorie data. Only Doctor Belsky has. And so I'm not quite sure about some of the data going into there.

But but I do know that the outcome of that was showing a significant reduction over the course of that to your timeline. Yeah. So, yeah, what would be perfect is if you got the rate of aging of point nine and then a year later your biological age was, you know, not a year older, but point nine over.

That would be yeah, that would be perfect. But you know, just for the listeners, you know, biomarkers of aging, even the Horvath clock, where it's 1.9 years, for any biomarker of aging, for that kind of variation is actually very good.

Other clocks, clock, other biomarkers that are sort of an R-squared of 0.4.5, which is still a very good one, like arterial stiffness or pulmonary function.

It's up closer to 0.7. I mean, you're talking about more like 3 to 5 years, which is frustrating when you're looking at, trying to see the effect of effective therapies.

Because, you know, you don't know for a couple of years. And unless what the trajectory is, patients can come back to me and said, I did my baseline telomere length with another doctor and then, you know, I started something and, you know, six months later, it was a year or better.

And I'm like, yeah, you did anything, you need, you know, there's, you know, at least three years of variation in that and you need more time points, for that sort of thing, which is, which is, you know, frustrating.

But with that rate of aging being a metric, really telling people, you know, whether or not their intermittent fasting or their, you know, 3 to 5 day fast or their NAD or whatever combinations of their that's what I like about it for clinical practice is we're doing stuff for our patients that they're it's on the cutting edge.

But if we have a metric that is quite well validated, like yours and in aggregate, whatever we're doing is moving it in the right direction, I think we can have good confidence and say that we're doing a good thing for the patient, particularly for, checking other biomarkers, and they're going in good directions as well.

They're pretty good for the newer things like, you know, the analytics therapies that some people are trying the, this that would be very interesting to see how those those are affect the extrinsic aging.

But for, you know, NAD therapies, hyperbaric oxygen, all these sorts of things would be very interesting to see, what's happening with the rate of aging.

And I'm sure we will be seeing that with if you work with your technology. Let's pivot for a second. If we have a little bit more time to talk about, your prediction of telomere length and, you know, where that's going since, I've been measuring telomere length in my practice for 14 years now, and, and it has the same issue of some variability.

But when you get a pretty good idea over time, what's, what's the what's the predictive, ability of that? And how did you go about doing that. So, so we base most of our work off of, Doctor Horvath algorithm, from, the what and sort of, several different, publicly visible data sets that have code methylation data entering or linked data, and then uses a sort of a the same type of, you know, regression modeling to then pick out the CPGs, which are predictive of telomere length.

And so this, is something we've been able to do and replicate, with some of our own data sets and telomere length, as it's a relatively common metric with a lot of the people that are doing our tests.

And so, what we've sort of found, particularly even with Doctor Horvath found, is that there is a relatively high correlation between telomere or I should say, estimated telomere length, via methylation and age.

So, in Doctor Horvath, particularly there was around a point seven, R-squared value for correlation to from telomere length estimation to age, which shows it to probably be a little bit more highly correlated with with certain types of outcomes. Right.

Or I should say more correlated with age. And then in addition to that, compared to traditional tumor testing in those subsets. And so that is also one of the caveats.

You know, there are a lot of different types of and methods of telomere testing. There are many types of subset breakdown things, like critically short term meal length or individual immune cell subtypes, human remains which weren't conducted in these studies.

And so so comparing, you know, the best thing to them are link testing to the best, the methylation testing, or methylation estimation via telomere testing.

It's still a hard thing to do. It's hard to compare those apples to apples. But but what they had shown essentially is that it was more predictive of some outcomes than traditional telomere length testing.

And that was particularly, congestive heart failure, cardiovascular disease, time till death. And then also, sort of correlation with smoking history.

And so, so that gave us reason to believe that we could also then train these methylation marks to predict the Mir length. You know, I think there's definitely more work that needs to be done at it, particularly looking at things like critically short telomerase length or, mute particular immune cell subset versions of telomere length.

But the idea is that hopefully we'll be able to train this even with the methylation prediction algorithm and do one test which can yield a lot of information, and be highly accurate versus just, you know, having to do multiple different tests to get the same amount of information.

Yeah. I mean, that's, that's, that's absolutely, you know, really fast if you know, I think that was, the correlations with Doctor Horvath, it was mostly, qPCR. Correct.

And so that's yeah, that's one of the I think, you know, less accurate, way, methods of doing is good for large observational studies in large batches.

Future Applications of Epigenetic Testing

But hopefully over time we'll be able to accumulate some data because all the filler lengths, measurements we're doing with, the physio agent and tree diagnostics will be able to get some blowfish, some blowfish data.

I hope the critically short ones is is really interesting. I don't think anybody really offers that clinically. Now, I mean, not critically. Short life length offers 20%, but, so, you know, we'll have to see whether that comes down the pike at some point.

Well, it's a, you know, it's a it's a it sounds like, you know, your working in the, in this field. And, you know, one of the best algorithms is it's probably that, the, the rate of aging, where do you see the field of, epigenetic, analysis, going at this point?

Where do you get. Definitely. Well, I would think I would feel remiss if I didn't go ahead and point out one of the probably the, if not the largest area already for epigenetic methylation, which is in, taking plasma from the blood and looking at, at a stage zero or stage one or stage two cancer detection, tissue specific origin.

Recently a test came out, just over the last few weeks, from Grail called Gallery, which is, able to to read over 27

different types of cancer. From from just a couple, you know, milliliters of plasma.

And that's really exciting because again, again, obviously cancer is one of the biggest, you know, risk burdens of everyone in these developing worlds.

And and to be able to detect it early is a huge clinical benefit. And so that is a huge area of epigenetic, evaluation, which is really exciting because what they can do with that cell free DNA that's found in the plasma is track it back to the tissue of origin.

And so you don't have just a, you know, marker that says you have cancer, you have it marking says you might have cancer in this tissue. And that can be very, very helpful, even just knowing where to look.

And so we're not doing much cancer work and, and the money going into that is, is pretty incredible. And really everyone should have a lot of hope for how we, we treat even cancer preventatively, I think, but in terms of, of areas that we're investigating, I think for us, we are looking to to build out some of those investigations into immune cell subsets and then also different types of diseases.

You know, things like, cognitive diseases that are associated with aging, like Alzheimer's or Parkinson's or, or even just dementia or cognitive impairment that happened with aging.

We're very excited to to learn more about those and, have some exciting things going on to look at those. We're interested in looking at cardiovascular disease metrics or, or even early diabetes markers that might encourage, you know, some type of lifestyle or medication change before we develop diabetes.

And so for us, I think that that the idea is to gather as much data as possible to find out where we see the highest correlations between methylation and disease, and then hopefully investigate those, with even further with a little bit more vigor and, to find out how we can predict different outcomes.

We're also very excited about things like, the exposome. Right, looking at, at, at a list of methylation values and being able to determine what type of exposures you've had across your life, to be able to then control for some of those exposures, and then also see if those exposures might impact the outcomes that we see for these people.

And so and then lastly, even things like farmaco epigenetics, everyone is very, you know, familiar probably with how your DNA might be, how you metabolize a drug or if you have side effects with a drug.

The same thing happens actually with epigenetics, where we might be able to even predict, you know, if you're going to have, side effects with metformin, or if you're going to have agency responses with metformin, which has actually already been published, that algorithm's already been published by a university in Sweden.

And so building and expanding on those, ideas of just how to better handle clinical practice and, and getting as much data as possible then to, to really build out the, the, the suite of this testing with there's so much information that can be found in the epigenome, that we think that this test can, can really reduce, costs on a lot of lab testing, but increase value.

And I think that that's where we see this being, hopefully going in the future and really every disease direction you can imagine, I would imagine would be impacted by this at one point.

Yeah. I'm certainly, it's allows you to personalized medicine, you know, tell your approach. I'm fascinated by the, the concept that you could predict, reaction to a medication, epigenetic leading, which maybe implies that if you make some changes, you can change the, your epigenetics and get rid of that, that, adverse reaction to it or lack of effective, effectiveness.

I'm sure those have trials have been done. Yeah, but but that that's really exciting as well. I mean, it's a it's an amazing technology that, you guys are at the forefront of and, and offering clinically now, is there, any thing you'd like to tell our viewers I know, you can tell, you know, where, how to get to the true diagnostic test.

Anything else you want to, you know, I, I would say that, this is a new and developing technology, and I want to give everyone also the skill set to adequately, differentiate if they're using a good platform. Right.

And I think that we've already mentioned some of the things there. I think that particularly you want to be able to use published algorithms. Right. Publish algorithms are very, very important because that's how we actually relate the change in those methylation marks to, to disease outcomes, right, via those studies.

And so I would highly recommend, you know, doing some work to vet if your algorithm has been published. I would also vet the tissue of collection. Right.

You know I we've mentioned we only do blood. Saliva is becoming a little bit more feasible due to some additional studies, but again, pay attention to the collection method.

And then and then also again, pay attention to the amount of data that you're getting, right? You know, as the amount of new developments in this field are growing, exponentially and being able to have a data set, which you can then look at for the future and, and beyond, I think is also important if you're just looking at, you know, 1000 to 2000 locations, then you might not have, the same information sort of across time, or even being able to look back ten years and say, hey, I had these markers, which I've been since changed.

And so so those are the three things that I would just, you know, hope every consumer or every practitioner would incorporate into their critical evaluation of any of these technologies as they continue to expand.

Yeah, I absolutely agree with all those. And those, they make good sense. I think it is, though, ready for prime time as a, as a clinical tool, particularly if you're applying therapies, that, you know, aren't looking at specific diseases, can't be measured, you know, for instance, we can't measure energy levels very easily right now.

But if you're raising it, if you're taking any of these supplement, which a lot of people are already, or taking metformin, even though you don't, you know, have a hemoglobin A1, C above, you know, even 5.6, you know, some people are taking it when they're even lower to know whether or not you're getting a benefit, particularly from this, you know, highly reproducible rate of aging, is a way for clinicians in, in my field and hopefully, you know, all primary care and other physicians at some point will be able to to use tools like this to, to give therapies a if they're needed be and know if they're working in an even as you said, predict beforehand who's going to have an adverse or beneficial response to it.

I think the future is really exciting, and I can see why every time I talk to you, you seem so excited about what you're doing. I know why now. So, thank you for coming on and giving a great introduction to this and getting into some of the the details of this stuff.

And, look forward to to talking to you again. Yeah. So thanks for having me on and wish you the best of luck with the other speakers. And, look forward to watching.