00:00 Sarah Crespi: Welcome to the Science podcast for June 5th, 2020. I'm Sarah Crespi. First up this week, staff writer, Meredith Wadman discusses a link between coronavirus, sex hormones and male pattern baldness. And it turns out that this link might be behind the higher numbers of men dying from the infection. Next we have researcher, Jason Chen. He talks about a system for tracking objects using DNA barcoded bacterial spores. You spray the spores on something like lettuce, and then if you ever need to know where that lettuce came from, perhaps if it was contaminated with dangerous bacteria, you can collect the spores and read out the DNA barcode. Now we have staff writer, Meredith Wadman. She wrote this weekend science on a potential source of the male bias we've been seeing in severe cases of coronavirus, even coronavirus deaths. Hi Meredith.

01:00 Meredith Wadman: Hi Sarah. How are you?

01:01 SC: I'm okay. I'm gonna say, okay for now. This has been a mysterious, but persistent skew in the number of deaths with regards to men. How big is this bias?

01:14 MW: Well, it's considerable and it's consistent from the very first days that we began getting reports out of Wuhan, China, men have been made sicker by COVID-19 and they died at higher rates than women from COVID-19. At the same time, children have been largely spared. And that too lines up with this theory that some researchers are putting forward that androgens, which are male hormones, may have a role to play in how badly people get sick from COVID.

01:46 SC: So there's this new research linking sex hormones and the severity of coronavirus. Where did this idea come from?

01:53 MW: At first blush it really landed with a paper that was published in Cell online in early March. And it designated or described a role for a particular enzyme that is bound in cell membranes called TMPRSS2. And it is an enzyme that cleaves the spike protein on the virus. And in doing that, it allows the virus to enter host cells. So it's important for viral entry. Now, a bunch of prostate cancer researchers looked at the Cell paper and said, "Woah, wait a minute..."

02:33 SC: We know that protein.

02:35 MW: "We know that protein." They knew the protein because years ago it was described as being a culprit in prostate cancer. This very same T-M-P-R-S-S-2 or TMPRSS2 enzyme in a mutated form, it was discovered early in this century, was responsible for about 50% of prostate cancers. So prostate researchers were intimately familiar with this enzyme. And one of the things they knew about it was that it was controlled by male hormones, at least in the prostate gland known as androgens of which testosterone is the most famous, actually increased the production of this enzyme in the prostate gland. When the enzyme was in a mutated form, it caused prostate cancer basically, in a simple definition.
03:30 SC: And so from that, you can take away that if you have more testosterone or other androgens, you're gonna have more of TMPRSS2. And so that's kind of the thinking behind this that you might have something that makes it easier for infection to take place.

03:44 MW: Absolutely. More TMPRSS2 on the cell membrane, more opportunities for the virus to knock and enter. It's almost that simple although, of course, it's way more complicated, but basically.

03:56 SC: Right. And there's some other interesting observations that you linked to this in your story. And one of them relates to male pattern baldness. How does that fit in?

04:06 MW: There's not a clear, scientific explanation for why it would be that a couple of studies in Spain have observed that men with male pattern baldness seem to be over-represented in male patients who are hospitalized with COVID-19.

04:27 SC: And that's not related to age?

04:29 MW: No. Interestingly, the peak baldness decade among these Spanish patients was in the 50s, whereas male baldness typically is most common in the 80s or even older.

04:43 SC: And there's a link between baldness and TMPRSS?

04:46 MW: Well, that's what's not entirely clear. What is known is that one very powerful male hormone named dihydrotestosterone or DHT for short, which is a derivative of testosterone, is abundant and thought to be causative of male pattern baldness when there's lots of it in the scalp. It's not causative on its own. It also takes some genetic predisposition. You have to have a couple of conditions, but one of them is high levels of this hormone, DHT. And that is the hormone that returning to the prostate, we know binds androgen receptor which in turn kicks up production of T-M-P-R-S-S-2, TMPRSS2. So there is a connection, but it just hasn't been born out in labs studies.

05:38 SC: That's all on the descriptive side like what we've seen, what we understand of this mechanism. What about the intervention side?

05:45 MW: A light bulb went off for the entire prostate cancer community, I think it's fair to say, when the Cell paper was published describing how TMPRSS2 helps the virus to enter cells. They knew that TMPRSS2 was regulated in the prostate by these male hormones called androgens. And they knew that there were several FDA approved drugs that are used as treatments in prostate cancer that beat down androgens. So it stood to reason that these treatments, although intended in the first instance for prostate cancer might help patients early in their infections with SARS-CoV-2.

06:26 SC: And is that something that has been observed out there already? People on these drugs having a different status of their infection?

06:34 MW: There's one key study that was published from the area around Venice in Italy, a region
called Veneto. Andrea Alimonti, an Italian scientist and his colleagues looked at data from men with prostate cancer, thousands of them who had been hospitalized in that region with COVID-19. And they discovered that the men who were on so-called androgen deprivation therapy these antiandrogen drugs, first of all, were much less likely to contract the virus than were men with prostate cancer not on these antiandrogen drugs. Then they went further and looked at the course in the hospital of the various populations of men, and discovered that those on the drugs did much better in the hospital, were less likely to end up in the ICU, and, in fact, not one of them died. Whereas there were deaths, mortality and real, what we call morbidity, really severe illness, much more commonly in the men with prostate cancer who were not on these antiandrogen drugs. So that was a powerful piece of epidemiologic evidence, and what it has led to is several clinical trials now being launched of these antiandrogen drugs in people hospitalized newly with a diagnosis of SARS-CoV-2 infection.

08:04 SC: So what would happen to a person that was given one of these antiandrogen drugs? What does it do to the body?

08:10 MW: They work at different points in what's called the androgen-signaling pathway, but basically the end result is that they drop your levels of testosterone or in some cases of this powerful testosterone metabolite called dihydrotestosterone. But the bottom line is that they dramatically reduce levels of testosterone or the effects of testosterone along this pathway, and in a sense, it's chemical castration. That sounds very brutal, but in point of fact, lots and lots of men with advanced prostate cancer are on these drugs, and the side effects might be something like hot flashes, impotence, slight growth of breast tissue called gynaecomastia. When you're coping with a really serious cancer, for some men, it's worth those side effects.

09:09 SC: That's a long-term course of the drugs.

09:12 MW: Exactly right. The course of treatment in these COVID-19 patients would be... Well, in one case it's one week that they're giving it in a trial at Johns Hopkins, and a trial at the VA in Los Angeles, and Seattle, and also Brooklyn is using an injection that lasts one month. So yes, it's drastic, but it's temporary and those side effects go away as the injection wears off. So you're buying yourself time, essentially, if this works as it's hoped to fight off the virus so that it can't enter cells so easily, so that your body's immune system can beat it back and other medications perhaps that you're taking can go to work. It's thought that by the time you're in serious trouble in the hospital, it's too late for these antiandrogens, if they're going to be effective, to likely work. It needs to be early in the course of disease as the virus is knocking for the first time at cellular doorways.

10:13 SC: We seem to have reasonable mechanism and some epidemiological evidence that this works. What are some of the end points of these clinical trials? What are they looking to see change with the administration of these drugs?

10:24 MW: We're hoping that, empirically that is, the proof is in the pudding, [chuckle] that these men will do better on androgen deprivation therapy as they battle COVID, and that you'll see less mortality, fewer people being put on ventilators, quicker recovery. These are some of the end points that the clinical trial people are going to be looking at as these trials move forward.
10:51 SC: Okay, Meredith, we've been talking about testosterone, male sex hormones, prostate cancer. What about women? Is this intervention, is this pathway active in women? Is this something that we're gonna be able to administer more broadly than just the male members of the population?

11:07 MW: Interestingly, women are involved in one clinical trial, at least the one at Johns Hopkins, where this antiandrogen drug also has a sort of pro-estrogen effect and estrogen is thought to help the lung heal from acute insults, acute injuries. So that's in part why they're including women in the trial. They're also including women because women have androgens, not near as many as men, but it's possible that there's some marginal gain if these drugs work really well in men that they might at least at the margin help women.

11:44 SC: Earlier when you said, "Everybody in the prostate cancer community perked up when they saw mention of this protein." Has this been kind of an unusual collaboration, because these people don't typically work on infectious disease?

11:58 MW: Right, yes, it has. And what's remarkable is how many people, scientists from different areas are pitching in. So you had, for instance, 600 prostate cancer researchers on a call last month to present the newest research on this possible antiandrogen effectiveness in COVID-19 patients. At the same time, you had stem cell biologists at UCSF who were running drug screens to see at the cellular level what might be happening on this antiandrogen pathway and what drugs might shut it down. And you have epidemiologists and dermatologists looking at baldness patterns, and it's just a sort of a team science where people are pitching in from all angles and fleshing out a really interesting picture very quickly and allowing, in addition, clinical trials to launch in weeks rather than the months or even years it would normally take to launch them. So I take hope from all of that.

13:04 SC: Alright, thank you so much, Meredith.

13:06 MW: You're so welcome, Sarah.

13:07 SC: Meredith Wadman is a staff writer for Science. You can find a link to her story and all of our COVID-19 coverage at sciencemag.org/podcasts. Stay tuned for an interview with Jason Chen about tracking everything from lettuce to sand using DNA-barcoded spores.

[music]

13:29 SC: This week Jason Chen and colleagues report on the development of a tagging system based on bacterial spores that can be implied to all sorts of things, from vegetables to sand. Jason is here to talk about how this system works and why we might wanna tag sand. Hi Jason.

13:44 Jason Chen: Hi, it's a pleasure to be here.

13:46 SC: What was the impetus for coming up with this system, what problem were you trying to solve with this bacterial spore tagging system?
13:54 JC: So we really wanted to solve the problem of object provenance. So, what object provenance means is to be able to determine the location history of an object. For example, where a produce has been produced and once it's travelled through the supply chain, being able to recover that information from the beginning.

14:17 SC: I've heard of provenance more in terms of art, so you wanna know if the piece of art is original and who's had it in their hands over the years. Why do you wanna track the origins of a lettuce and where it's been along its route?

14:30 JC: Every year, there's almost 50 million cases of food-borne illnesses, and it takes usually few weeks to a couple of months to figure out where the actual outbreak has occurred. What we wanted to be able to see if we can develop a method to quickly tag some of the lettuce that's been produced from its originating farm to the end of a kitchen table where you have a lettuce on the table.

14:57 SC: You use bacterial spores to do this. What's so good about using these as carriers of that information?

15:04 JC: Spores are basically very hardy, nature's design of survivability. So from the ancient Egyptians, there are seeds and spores that they've cultured and that we can recover still nowadays from archeological findings and they can still germinate. So they've been through thousands of years, basically in hibernation and they can still be... They're still alive.

15:31 SC: Yeah, what exactly is a spore in this context?

15:33 JC: So a spore is basically when a bacteria or a yeast, when it senses a lack of nutrients around its surrounding, it starts to form a shell around itself in order to go into a sort of hibernation mode. Once they sense nutrients again, then there're these germination receptors that sense water or nutrients and they start to restart transcription of different genes to start germination.

16:01 SC: In the paper, you used a number of different methods to inactivate these spores to prevent this germination from happening. And you did things like oiling or removing key genes that the bacteria can use to turn themselves back on. Why was that important to do?

16:15 JC: It was important both from a bio-containment side, we didn't want our spores once we put into the environment to spread and overtake the microbiome of the place that we put them in and we show that one, that our spores don't grow for up to three months in our experiments, as well as we've done a real life experiment where we put the spores in a protected outdoor environment for up to six months and we don't see any spread.

16:48 SC: Yeah, you don't wanna tag something and have the tag just continually replicate and cover everything or outcompete the natural microbes that are present in the environment.

16:58 JC: Exactly.
17:00 SC: Let's talk about the tagging part of this. So you have these spores, but what about them makes them unique, how can they serve as a tag?

17:07 JC: We relied on DNA barcoding. They're quite small barcodes of about 38 DNA base pairs. We designed them such that they're different enough so that we can design up to billions of unique barcodes. We designed these tandem barcodes such that one of the barcode is specific to a farm, another one is specific to the region, basically how zip code works. We can even design a third one or a fourth one, we can envision for future iterations to have city or a state barcodes, we can screen for different states first and then go into different cities.

17:48 SC: Once you want to read a tag on say, a piece of lettuce, how do you get that barcode information out?

17:55 JC: Once we sprayed the barcodes onto the lettuce, we just take a piece of the lettuce and we extract DNA from it, and we just run our detection assay on it.

18:07 SC: And that's like a probe that's specific for a barcode?

18:10 JC: Exactly, so we have CRISPR RNAs that are specific to the barcodes that we put into our reaction and the Cas13 or the CRISPR protein will bind to and activate the signal.

18:24 SC: What does the result look like? It's not gonna give you a set of numbers, is it just a bunch of blotches?

18:30 JC: Whenever it's positive, then you see a yellow signal and whenever it's negative, it's gonna stay blank.

18:36 SC: So when you say positive, you're saying it's matching a barcode that you're using as the probe or just saying, "Yeah, there's a barcode here?"

18:41 JC: When there's a specific barcode in your reaction.

18:46 SC: So we're talking about putting spores on people's food, is that something that we eat all the time, is that everywhere around us, anyway?

18:53 JC: Yeah, surprisingly, we eat all kinds of microbes all the time. For example, in yogurt, we eat lactobacillus. When you buy your meat under shelf at the supermarket we often don't realize that producers spray phages onto it to prevent bacteria. [chuckle]

19:12 SC: So a phage is a virus that kills bacteria. That's amazing, I didn't know that.

19:17 JC: Yeah, that's right. So we eat all of these all the time. We foresee our barcodes, we put in BT, bacillus thuringiensissirens and BT has been used as a natural biocide on crops already. It's been FDA approved and now, actually organic farmers instead of using chemical pesticides, they use BT because it's natural and it doesn't adversely affect humans and it prevents pests from
SciencePodcast_200605

growing.

19:49 SC: What kinds of things did you stick the spores to in this study?

19:52 JC: We tested different surfaces. Wood, carpets, sand as well.

19:58 SC: Why did you wanna put it on sand?

20:01 JC: Sand because it's relatively cheap to get, and it's also relatively similar to where we actually walk. And sand was one of the easier medium to collect our samples from.

20:16 SC: Okay. How long did the spores stick around on these various surfaces? You said wood, sand, I'm assuming some kind of foods.

20:25 JC: We did some experiments where we blew wind every week for up to three months. We simulated water by draining the sand with the hose. We showed that there were no effective changes to our environment.

20:40 SC: So months, these spores stuck around on the surface.

20:43 JC: Yep. Even though they stick there for a long time, it's relatively easy to get rid of them as well, like 10% bleach. You can just lice them.

20:53 SC: That's really good to know.

20:54 JC: Yeah.

20:54 SC: I was definitely gonna ask you how to get these off of things. I did find it surprising considering how long these tags, these spores seem to persist. They also transfer. You were able to show that if you walk over the sand or a floor, that it would get on shoes. So how much transfer did you see?

21:12 JC: Just by stepping a shoe onto a inoculated area is enough spores to detect it. And we've done experiments where we tested once, spores were transferred onto the shoe, we walked it around for various amount of time, up to four hours. And the spores are pretty sticky. And that after four hours, we only see two-fold decrease in the spores that are stuck on the shoes.

21:43 SC: So why would you wanna know whether or not the spores transfer to your shoes? Is that because somebody's gonna work at one of these farms and end up with tags all over their feet?

21:52 JC: Our purpose was more to apply these spores onto detecting lettuce provenance, but we also wanted to test the breadth and the range of things that this technology is capable of. And it was easier to have someone wear a shoe and then move it around. It was not that we wanted to track people per se, but it was just to see if we can have objects travel through different areas, and see if we can recover the spores back from them.
22:22 SC: So you could do this. Like I could, instead of micro-chipping my dog, I could give my dog some spores that have a barcode on them?

22:28 JC: That's possibly [chuckle] one way to use it, but we wouldn't advise you to do that. [chuckle]

22:36 SC: It's a lot of work, right, compared to just having a little chip reader in every vet's office?

22:40 JC: Exactly.

22:42 SC: And you can get this stuff off? As you mentioned, a bleach solution will take it off your shoes, or I guess your dog, if you wanted to?

22:50 JC: Exactly.

22:51 SC: Well, speaking of transferring, what happens if the lettuce mingles, if a banana touches lettuce? Are you gonna get cross-contamination of these bar codes? Is it gonna mess up the signal that you're looking for when you're tracing the provenance?

23:05 JC: Actually, one of the observations that we saw was that when you spray it, the barcodes onto leaves or vegetables, the transfer rate is much less than when you spray on sand and have a shoe stick on there. Here, we just speculate perhaps the spores get stuck into the micro-structures of the leaves. Nevertheless, we still see some transfer when we put two leaves that are barcoded with different barcodes. But that didn't prevent us from being able to recover the original barcode.

23:08 SC: Another experiment that you did involved going to the grocery store, taking home some vegetables, and looking for this BT on something that's already out there for sale, and seeing how it survives various processes that it might undergo in the kitchen. How did that work? What did you see when you ran those experiments?

23:40 SC: We just went to the supermarket and bought various brands of greens and produces and we had primers designed to specifically detect some genes from BT. And unsurprisingly, we were able to detect BT. And so, that also gave us a good indication that things that are sprayed at the beginning can be recovered through the entire produce market.

24:01 JC: What happens if you cook it?

24:31 JC: Even after we cook it, we could still detect some of the spores. So if you get sick after cooked food, we can still detect the barcodes from there.

24:41 SC: Yeah.

24:42 JC: So it's quite robust.
24:43 SC: Thank you so much, Jason.

24:44 JC: Thank you. It was a pleasure to being here.

24:46 SC: Jason Chen is a PhD student in the systems biology department at Harvard Medical School. You can find a link to his paper and a related commentary piece at sciencemag.org/podcast.

[music]

24:58 SC: And that concludes this edition of the Science podcast. If you have any comments or suggestions for the show, write to us at sciencepodcast@aaas.org. You can listen to the show on the Science website. That's sciencemag.org/podcasts. There, you'll find links to the research and news discussed in the episode. And of course, you can subscribe to the show anywhere you get your podcasts. This show was edited and produced by Sarah Crespi, with production help from Podigy, Meagan Cantwell and Joel Goldberg. Jeffrey Cook composed the music. On behalf of Science magazine and its publisher, AAAS, thanks for joining us.