Yunxiao Zhang: Thanks organizers for the fantastic, symposium today. I'm Yunxiao currently a postdoc working at Scripps in Dr. Ardem Patapoutian's lab. I'm finishing up my postdoc now and just soon going to establish my own lab in China. Today, I'm going to talk about the mechanosensory control of pregnancy and parturition. The mechanical forces is really obvious throughout the pregnancy and labor process as we easily record all these different mechanical stretches and mechanical forces throughout the process.

Yunxiao Zhang: There has been a lot of early studies suggesting that the mechanical forces are playing some regulatory roles at different stages of the whole process. But directly pinpointing the exact molecular mechanism for how our body senses the mechanical force and what exactly roles the mechanical forces have on the overall reproductive system has been difficult as there are so many different signaling mechanisms that can be affected by mechanical stretch, but they also tend to respond to a bunch of other biochemical signaling, making it very hard to isolate the specific effect from the mechanical force alone.

Yunxiao Zhang: That's why in my study, I turned into the mechanically activated channel, the PIEZO channels, which respond to mechanical stretch by simply opening to allow the calcium to enter the cell. Despite all the extensive studies, we have not found any physiological ligands that can directly activate PIEZO so that the PIEZO activity is almost always associated with mechanical forces. With that in mind, we checked the extension patterns of PIEZO in the tissues.

Yunxiao Zhang: Shown here on the left is in situ hybridization for the PIEZO transcripts in the human myometrium. As highlighted here that the PIEZO1 is highly expressed in the smooth muscle cells within the myometrium. We also checked the mouse uterus tissues at late pregnancy and all the expression patterns are highly similar between human and mouse. On the other hand, PIEZO2 as the homolog PIEZO1 is minimally expressed within the uterus itself, but it is known to be expressed extensively in the dorsal root ganglia where the sensory neurons reside.

Yunxiao Zhang: We checked the PIEZO2 positive fibers in the mouth and found that the PIEZO2 positive fibers form a dense meshwork around the cervical region, which would be poised to sense the mechanical stretch during the labor process. The contribution of PIEZO2 positive fibers with the uterine horn is very limited. With these distinct patterns of PIEZO1 and PIEZO2 we believe that both can potentially contribute to the pregnancy and labor process. Therefore, we use mouse genetics to knock out both of them to say the phenotypes associated with PIEZO lead to loss of function.

Yunxiao Zhang: As I mentioned before, since PIEZO seem to be very specific to the mechanical simulation, therefore, any differences from a loss of function, mouse genetics with PIEZO1, PIEZO2 can be almost directly associated with a defect in the mechanism. We tract the mouse of the knockout animals throughout their pregnancy and labor.

Yunxiao Zhang: Their pregnancy can proceed smoothly without much complications as the overall development of the embryos seems to be comparable between control and knockout animals. But the real defect occurs when the animals go into labor. As shown here in the charts, the dark and light bars on the top suggests the dark and light cycles of the day. Each of the cell indicates one of the pregnant animals, and each of the little green bar indicates the delivery of one pup. Showing at the left is it's a group of controlled animals.

Yunxiao Zhang: You can see that all green bars are tightly clustered together, suggesting that the delivery of the pups have really quick succession with one almost immediately after another which is typical of mouse with the pregnancy of usually 8-10 animals. But on the right side is a double knock PIEZO1 and PIEZO2 double knockout animals. You can see that the green bars are more space a part suggesting that there tends to be some long pauses between the delivery of the animals, which indicates a slowdown of over labor process.

Yunxiao Zhang: Another difference we see here is the timing. As we can see that the control animals, most of them deliver the pups at the dark bar suggesting in the dark cycle or right at the time of dawn, so when the light just turns on, which is what we know about the normal scaling rhythm of the mouse. But in the double knockout animals, the initiation of the labor with the first arrival of the first pups seem to be pushback towards the day, and some of them even start labor in the afternoon time, which is very unusual.

Yunxiao Zhang: Shown here is some quantification as I described earlier, that the intervals between the pups for these different animals seem to be much longer in the double knockout animals compared to the control animals, suggesting a slowdown of the labor process, and the initiation as indicated by the arrival of the first pup also tends to be pushback in the double knockout animals.

Yunxiao Zhang: These two different observations, we also did a measurement of intrauterine pressure which indicates the uterine contraction during this process, we also saw an overall decrease of the intrauterine pressure at the time of labor, suggesting weaker labor contractions, but we did not see changes in the pre-labor weak contractions, and also contractions after the completion of the labor.

Yunxiao Zhang: With all these different observations of phenotype, we also want to answer how this came about, what exactly is contributing to all these observations. Based on the extraction patterns, we have seen that the PIEZO2 seem to be forming the nerve fibers wrapping around the cervical region, which is the right place to sense stretch at the time of foetus passage. But PIEZO1 seem to be express with the smooth muscle cells.

Yunxiao Zhang: As we know that the pre labor contractions which is much weaker compared to the labor, but those often occur several days before the time of labor. Those contractions, although vague, they are strong enough to activate PIEZO1. Therefore, PIEZO1 is likely to be activated long before the initiation of labor as a prolonged activation of PIEZO1 allows calcium influx into the cells, which, as known from many other different systems, is often associated with long-term remodeling, and therefore, we check the gene expression within the uterus and late pregnancy.

Yunxiao Zhang: From the single cell sequencing, we have noted the upregulation of several contracted associated genes is impaired in the PIEZO knockout animals, and we confirm this with in situ hybridization shown here of the mouse uterine sections as gestation of the 18.5, indicating that the gap junction, the Cx43 transcript is down regulated in the double knockout animals, which is one of the contracted associate genes and associated with the uterine contractions at the time of labor.

Yunxiao Zhang: With all these observations, I have shown that the mechanism mediated by the PIEZO seem to be really is a strong contributor to the uterine contractions at the time of labor, and PIEZO is only the first step into the study of the mechanical cessation during the whole pregnancy and parturition steps. There are a lot of other false sensing mechanisms still yet to be studied and the take home message from this is mechanical force, although we cannot see it, but we all feel it.

Yunxiao Zhang: It is definitely one of the new angles we should look into about how the overall pregnancy and parturition process is regulated. At the end, I want to thank the Patapoutian Lab for the support and thank my collaborators from WashU medicine. At this time, I'm wrapping up my post doc. I'm going to soon establish my own lab at the Westlake University in China. Now I'm happy to take your questions. Thank you.

FEMALE_1: [APPLAUSE] Thank you so much for this amazing work.

FEMALE_2: Do you know if the PIEZO gets expressed in the myometrium or cervix the same throughout the pregnancy or is it something because, for example, oxytocin receptors, we know that they change during gestation in both rodents and humans?

Yunxiao Zhang: We checked the PIEZO expression between the non-pregnant state versus the pregnant state. In the smooth muscle cells, there is a very mild increase, but not very significant. Also during the different stages of the extra cycle, there is definitely some fluctuation of PIEZO expression within smooth muscle cells, but the changes is always in twofold. It's a relatively vague change.

Yunxiao Zhang: But one thing that I did not cover in my study, but we do see is the changes as the expression of the PIEZO within the epithelial layer of the tissues. There's definitely a very interesting expression pattern as within the cervical region. There seems to be a lot more the PIEZO2 expression within the epithelial layer of the cervix, but within the uterus horn, the expression of the PIEZO is mainly the PIEZO1.

FEMALE_3: Hi there. Great talk. We know clinically that some patients are more likely to have a failed labor or labor arrest, a failed induction of labor, and one of the main risk factors for that is patients with obesity. I noticed some of your collaborators at WashU, but I was wondering if you have any knowledge or understanding of expression and function of PIEZO channels and obesity?

Yunxiao Zhang: From a separate study, we were studying the adipose issues on the high fat diet. We did not really see much of the extraction change of the PIEZO itself. But what we do know is because the force sensing mechanism of PIEZO1 is the primary mechanism is so called force from lipid, that is, it senses gestation from the lipid bilayer. We do see very well from individual studies that saturated fatty acid can change the membrane stiffness and that directly affects the sensitivity of PIEZO1.

Yunxiao Zhang: Basically, when you have more saturated fatty acid in the lipid bilayer, then the PIEZO1 is much harder to open that is, and with polyunsaturated fatty acid, it has the opposite effect on PIEZO1. This lipid compensation change in the cells can be another subtle way to modulate the overall sensitivity of the channels.

FEMALE_2: I have one more question. Who was in the lab, when she studied the PIEZO in the bladder, for example, so they did specific knockout of the neuronal PIEZO sensory neurons, and then, of course, in erythelium and then both. I was just curious because you alluded to the fact that there's a lot of PIEZO in the nerves around the cervix. I don't know if it's possible to do the same that she did for the bladder?

Yunxiao Zhang: I did not show that part of the data. I did the sensory knockout PIEZO1 in the uterus itself versus PIEZO in the sensory neurons so the phenotype is actually very different. Knockout of the PIEZO in the sensory neurons cause delayed onset of the labor. Essentially, it mostly recapitulated what I saw with the double knockouts that the first part usually arrives in the day. Some of them even arrive in the late afternoon.

Yunxiao Zhang: But the knockout of PIEZO within the uterus compartment itself with the projection receptor, it only cause a mild decrease in the overall uterine contraction itself. With the knockout from the sensory neurons, I also see a small decrease in the overall uterine contraction at the time of labor. But when we combine the knockout of both the uterus itself and the sensory neurons, then the phenotype becomes much stronger.

Yunxiao Zhang: That's when we begin to see a slowdown labor that's an increase in the intervals between the parts, but with a single only in the uterus or in the sensory neurons, we do not really prolonged intervals between the parts. I think a part of this is also because unlike humans, we have a really big head and also the very narrow pelvic structure.

Yunxiao Zhang: That the overall parturition process is much harder in human compared to mouse so that in mouth with all these defects, same as the overall labor process is only mild affected, but if we really translate to humans especially with epidural analgesia, if we really hit the sensory neural hard so that we even block the motor functions, we definitely know that from the clinical report that's

going to be really detrimental for the overall delivery process.

FEMALE_2: Thank you.

Yunxiao Zhang: Thank you [APPLAUSE]

[MUSIC]