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## CASR and ADH1: Integrating Biology and Genetic Screening

### Announcer:

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### Dr. Schweiger:

Hello, everybody. Thank you for joining us today. My name is Michelle Schweiger. And I'm the Director of Pediatric Endocrinology here at Cedars Sinai Medical Center. And it's with great pleasure to have Dr. Michael Levine, from Children's Hospital of Philadelphia as our speaker here today as well.

### Dr. Levine:

Greetings from Philadelphia, everybody. It's a pleasure to be here. And I look forward to our discussion, Michelle.

### Dr. Schweiger:

And our topic for today is the biology of calcium-sensing receptor mutations.

So, Dr. Levine, we know that the calcium-sensing receptor is a member of the super family of heptahelical transmembrane receptors that are coupled to G protein-dependent signaling pathways. Can you please tell us about the calcium-sensing receptor, where it is expressed and how it functions?

### Dr. Levine:

That's a great question, Michelle. The calcium-sensing receptor is a very large protein with over 1,000 amino acids. It is, as you point, out a G protein-coupled receptor, so it's part of his super family of receptors that bind ligands, such as TSH, PTH, ADH, and many other hormones and neurotransmitters. So, the signaling mechanism of the calcium-sensing receptor to use a G protein as a signal transducer is a well-known paradigm in endocrinology and in many other areas of biology.

The calcium-sensing receptor exists as a dimer, and it's ubiquitously expressed, although it's important to note that it's most highly expressed in the parathyroid cells, particularly the chief cells, as well as in the kidney, particularly along the thick ascending limb. The native ligand for the calcium-sensing receptor is, no surprise, calcium.

And this was really a high watermark in the field of bone and mineral metabolism when, a number of years ago, Ed Brown and his colleagues identified the calcium-sensing receptor as a receptor for calcium. And this expanded the repertoire of biological processes that we associate with calcium. Not only is calcium important in neuromuscular physiology as a cofactor for many enzymes and clotting factors, also important for stimulus contraction coupling in muscle cells, as well of course, as being involved in calcification of the skeleton.

But now we can consider calcium as a ligand and as an endocrine hormone. And binding of calcium to the calcium-sensing receptor leads to activation of a number of G proteins, most importantly, Gq and G11, which coupled to phospholipase C type beta. And activation of phospholipase C leads to hydrolysis of membrane-bound phospho inositol 4,5 bisphosphate, which yields two important

products, diacylglycerol as well as IP3. And the diacylglycerol activates protein kinase C, which then activates an entire repertoire of signaling events that are coupled to protein kinase C. And the IP3 will bind to specific receptors on the endoplasmic reticulum that allows stored calcium to be released into the cytosol, thereby increasing cytosolic calcium levels. So, binding of the calcium-sensing receptor to its receptor outside the cell leads to increases in cytosolic calcium inside the cell, but that calcium comes from storage pools within the cell. And as cytosolic calcium levels increase within the parathyroid cell, there is inhibition of production of PTH, as well as inhibition of PTH secretion.

Now, in addition to all of these, I think, marvelous signaling properties, it's important to note that this is a very different mechanism by which calcium is affecting release of a hormone. In the parathyroid cell, increased cytosolic calcium decreases secretion of PTH, the hormone that's stored in the cell, whereas in nearly every other cell, an increase in intracellular calcium leads to an increase in secretion of the stored hormone. And the basis for this contrasting physiology, the parathyroid versus the rest of the endocrine world, remains an unsolved mystery.

Now, the calcium-sensing receptor on the parathyroid chief cell provides an exquisitely sensitive mechanism for the parathyroid cell to sense the level of extracellular ionized calcium. And there's a sigmoidal curve with a very steep portion that describes the relationship between increasing levels of extracellular ionized calcium and decreasing secretion of PTH. And the recognition of the central role of the calcium-sensing receptor in controlling not only PTH production, but also PTH secretion, really, I think encouraged study of the calcium-sensing receptor as a therapeutic target for manipulating or controlling the release of PTH from the parathyroid glands. So, this opened up a whole new field of pharmacology with the calcium-sensing receptor as a target molecule.

The other thing we should remember is that, in the kidney, particularly in the thick ascending limb of the nephron, the calcium-sensing receptor can inhibit reabsorption of calcium, sodium, and water. So, we have to think of it as more than just inhibiting the reabsorption of calcium. And together, if we think of the two major targets, the calcium-sensing receptor in the parathyroid and in the kidney, the activation of signaling in these two tissues, explains the low level of PTH, the decrease in secretion of parathyroid hormone with hypocalcemia and the increase in urinary calcium excretion, both can be completely explained by the gain of function in the calcium-sensing receptor.

So, the excess of urinary calcium excretion in patients with ADH1, which is a hallmark of this condition and differentiates it from other forms of hypoparathyroidism, really represents a sort of double whammy, if you will, the loss of PTH, which can increase reabsorption of calcium, and the activation of the calcium-sensing receptor, which now decreases calcium reabsorption. So, the loss of calcium in the kidney is the result of two different lesions.

**Dr. Schweiger:**

Thank you. That was really very helpful. So, some of the key points on that is the intricacies of the calcium-sensing receptor, both on the kidney and in the parathyroid gland. And just kind of any kind of gain of function mutation or loss of function mutation can really kind of alter the calcium secretion and PTH function. Thank you.

**Dr. Levine:**

Exactly, exactly.

**Dr. Schweiger:**

So, the next question that I had is, we know that the calcium-sensing receptor is also implicated in familial hypercalcemia hypercalciuria. Tell us how this same receptor can be involved in ADH1 and familial hypocalcemic hypercalciuria, which are remarkably contrasting disorders of mineral metabolism?

**Dr. Levine:**

That's a great question, Michelle. This is an example of what I think is an exceedingly pleasing bit of biological symmetry. And there are many other G protein coupled receptors, as well as G proteins that can have mutations that lead to either a gain or function or loss of function, and which result in contrasting phenotypes, which I think is really quite remarkable. I'm always reminded of Gs alpha, where the loss of function leads to pseudohypoparathyroidism and resistance to PTH and TSH, and then McCune-Albright syndrome, where the gain of function can lead to overactivity in some of the same tissues that are affected by the hormones in which this resistance and pseudohypoparathyroidism. So, this is a well-worn paradigm.

In FHH, familial hypocalciuric hypercalcemia, there is a loss of function mutation that leads to an inability of the parathyroid cell to sense extracellular calcium. And in this scenario, the parathyroid cell thinks that the serum calcium level is too low, and this leads to increased secretion of PTH and hypercalcemia. In the kidney, the loss of activity of the calcium-sensing receptor leads to increased reabsorption of calcium, so there's very little calcium in the urine in patients who have FHH. This whole mechanism is amplified in patients who have neonatal severe hyperparathyroidism, due either to dominant negative mutations in an allele of the calcium-sensing receptor, that leads

to a loss of activity not only in the pathogenic allele but also in the protein made by the normal allele. Or even in those cases where there are biallelic mutations. Here, the complete loss of calcium-sensing receptor proteins leads to an inability of the parathyroid cell to detect calcium. And babies are born with very high levels of PTH, life-threatening hypercalcemia, severe metabolic bone disease that often impairs their ability to breathe because of the effect of high PTH on the ribcage. So, FHH and neonatal severe hyperparathyroidism represent one end of the spectrum of calcium-sensing receptor disorders. In this case, there is a loss of function.

And in the other case, ADH1, we have the gain of function. And again, the target molecule here is the calcium-sensing receptor. And this is led and encouraged work to use the calcium-sensing receptor as a therapeutic target. And we know that using calcium mimetics type 2 allosteric regulators of the calcium-sensing receptor can, in many cases, reduce secretion of PTH, normalize serum calcium levels. And as we'll hear in a little bit, the calcilytic drugs have also been used now in preclinical and emerging clinical studies to decrease sensitivity of the calcium-sensing receptor, shift the curve to the right. And by reducing activity of the calcium sensing receptor, you can actually increase secretion of PTH, thereby normalizing serum levels of calcium.

**Dr. Schweiger:**

Thank you. The next question I had was the discovery that mutations that activate the calcium-sensing receptor are the basis for ADH1 provides us with a molecular testing strategy. Please tell us how you screen for mutations in the calcium-sensing receptor, and how genetic testing can be used to diagnose ADH1?

**Dr. Levine:**

That's a great point, Michelle. And I think that the availability of molecular genetic testing has really changed the way that we approach the diagnosis of complex and difficult clinical disorders these days, particularly in endocrinology, where we have been fortunate to elucidate the molecular cause of so many of the diseases that we treat. And using a molecular genetic strategy, either knowing a gene or using a panel of genes can enable us to quickly determine what the root cause of the disorder is, and then begin to develop a therapeutic management plan based on knowledge of the underlying genetic defect and the pathways that are affected.

For the calcium-sensing receptor, over 400 mutations have been identified in patients with familial hypocalcemic hypercalcemia, FHH, or in patients with autosomal dominant hypocalcemia type 1, ADH1. And the vast majority of these mutations are small point mutations that can be identified by sequencing strategies such as Sanger sequencing, or the newer technologies called next generation sequencing.

So, I think an early appreciation that genetics can provide us with the proper diagnosis in patients with hypoparathyroidism, really means that we want to be doing genetic testing earlier rather than later, once we've made a diagnosis of hypoparathyroidism. And because ADH1 is a condition that reflects a germline mutation in the calcium-sensing receptor, the mutation will be present in all the cells of a patient. This means that the patient may have inherited the mutation and certainly means the patient can transmit mutation. And again, because it's a germline mutation, and the mutation is present in DNA from all cells, you can use DNA from a variety of different cells to do the analysis. So, cells that are present in saliva, or even in peripheral blood samples, are very useful sources of DNA for the kinds of analyses that we need to do in order to identify the mutation in the calcium-sensing receptor.

Now, I should mention that Calcilytx and PreventionGenetics have formed a partnership to provide no-cost sponsored genetic testing for patients in the United States and Canada, who have a diagnosis of hypoparathyroidism or hypocalcemia. That's non-surgical in its basis. And this free testing uses next generation sequencing to interrogate a panel of nearly 30 different genes, which includes the calcium-sensing receptor. And the testing is extremely useful. It's sensitive, it looks at all the exons in the calcium-sensing receptor, as well as 10 to 20 bases in the introns that flank each exon. And the results are reported as a standard clinical report with an interpretation provided by PreventionGenetics, that is consistent with ACGM guidelines.

Using this, one can identify mutations in patients with ADH1. And not only is this important for them guiding the appropriate treatment of patients with ADH1, but it also now enables family testing to occur. And although 80% of patients with ADH1 will have an effective relative who has ADH1, only about 25% of patients with ADH1 are identified because of family screening. So, once you've identified the genetic defect in the calcium-sensing receptor, it's easy then to screen all the other first-degree relatives with a serum calcium and a genetic test, looking for the mutation.

This is also critically important because there's a very long gap in diagnosis of ADH1. The median age which patients with ADH1 are diagnosed with hypoparathyroidism is 4 years. But the median age at which ADH1 is diagnosed is 25 years of age. So, we can close this gap by going to earlier genetic testing in order to identify patients with calcium-sensing receptor mutations.

And I would add to that, that the other important part of doing the family screening is that many patients will be asymptomatic, and they would not have been identified as having this condition without doing some kind of family screening. And you know, people ask the question all the time, why screen asymptomatic patients? How do you make the asymptomatic patient feel better? And it turns out as

with many other chronic endocrine disorders, once you treat a patient, they realize that they were not asymptomatic, and they begin to feel much better with treatment. So, I think that provides yet another incentive for us to identify patients with ADH1 who may consider themselves to be asymptomatic because treatment can make them feel better.

**Dr. Schweiger:**

Sounds good. So, it sounds, you know, that we need to have a heightened awareness not only for our symptomatic patients with autosomal dominant hypoparathyroidism, but also being on the lookout for these asymptomatic patients that might not be so readily available as far as diagnosis is easy to tell. Because in these patients, they also have an increased risk for basal ganglia calcifications, seizure disorders, and nephrocalcinosis. And so, if we can, you know, evaluate these patients early and get them diagnosed, we can help prevent some of these complications.

**Dr. Levine:**

Correct.

**Dr. Schweiger:**

Thank you, everyone, for joining us today. Wishing everyone a very great day from here in sunny California. Thank you.

**Dr. Levine:**

And bye-bye from Philadelphia.

**Announcer:**

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