Zebrafish as a Model System for the Study of Severe $Ca_v2.1(\alpha_{1A})$ Channelopathies. Tyagi, S,, Ribera, A.B., and Bannister, R.A. *Frontiers in Molecular Neuroscience*. February (12):329.

Variants: G232V, L356V, A405T, A715T, R1350Q, I1342T, I1357S, V1396M, E1755G, E1761K, R1673P, Y1667N.

Diagnosis/symptoms: FHM1, EA2, congenital ataxia, global developmental delay, hypotonia, dyskinesia, cerebellar atrophy, dysmetria, cognitive impairment.

This paper reviewed the $Ca_v2.1$ voltage-gated Ca^{2+} ion channel, known diseases linked to the channel, and new mutations that require further functional studies. It also proposed using zebrafish as an *in vivo* (whole organism) model to study the biological mechanisms behind *CACNA1A* mutations and as a method to screen for useful drug therapies for $Ca_v2.1$ channelopathies.

The *CACNA1A* gene encodes for the α_{1A} (alpha-1A) subunit of the $Ca_v2.1$ calcium ion channel expressed in neuromuscular junctions (where muscles and nerves meet) and central synapses (between neurons). While alpha-1A is the primary subunit, it works with two other $Ca_v2.1$ subunits β and $\alpha_2\delta$ (beta and alpha 2 delta) to form a fully functional ion channel. The alpha-1A contains four repeated domains (RI-RIV), each with six membrane spanning subunits (S1-S6). Subunits S1-S4 act as the voltage sensors that detect the changes in ions at the membrane and signals for Ca^{2+} to move into neurons. S4 is the primary sensor. S5 and S6 make up the actual pore, or channel in the membrane. There is also a large extracellular (outside the cell) loop called the P-loop between each S5 and S6 subunit that acts as a selectivity filter to only allow Ca^{2+} though.

Two diseases previously linked to *CACNA1A* variants are FHM1 (Familial Hemiplegic Migraine Type 1) and EA2 (Episodic Ataxia Type 2). EA2, characterized by sudden ataxic attacks, nystagmus, and vertigo, has been linked to loss-of function (LOF) variants. Functional studies looking at channel activity confirmed this. These variants have been mapped to the P-loop, S5, and S6. FHM1 is characterized by weakness of half of the body for long periods of time, brought on by migraines. Most of the variants linked to FHM1 are classified as gain-of-function (GOF) variants, meaning the ion channels are overactive and allow more Ca^{2+} into neurons. Functional data also confirmed this. Many FHM1 variants are found in the loops between S3-S4, S5-S6, or in S4. The authors emphasized a new class of *CACNA1A* variants, identified by whole exome sequencing, that exhibit the migraine and ataxia phenotypes seen in FHM1 and EA2 but with more severe developmental components. These variants are also located in different locations than the FHM1 and EA2 variants, suggesting they may have a different molecular disease mechanism. The following is a summary of some of the new class variants from reviewing the scientific literature.

A405T was found in a patient showing cerebellar symptoms of ataxia, dysmetria (inaccurate voluntary movement), and hypotonia (low muscle tone). It is located in a loop between the repeated domains I and II. Based on previous clinical studies and work in cell lines, the variant is thought to interrupt the interaction between the alpha-1A subunit with the beta subunit, suggesting a LOF effect. This might prevent the entire $Ca_v2.1$ channel from moving to

the cell membrane and decrease the number of functioning channels. Another study suggested that A405T might disrupt neurotransmitter release by interfering with interactions of the channel with other proteins required for their release.

R1350Q was studied in a patient showing cerebellar ataxia, development delays, and general dyskinesia (uncontrolled involuntary movement). This mutation is located in S4, the main voltage sensor subunit, and functional studies in mice suggested it was a GOF variant. There was a hyperpolarization shift in activation, meaning the calcium channel was activated sooner than normal. However, this was in conflict with other functional studies in cells expressing this variant that showed a loss of channel activity, or LOF effects. The authors only commented that further work needed to be done to resolve this.

I1342T and **V1396M** were found independently in patients showing congenital ataxia, hypotonia, and intellectual disability. I1342T is thought to impact the movement of the S2 subunit during channel activation, but functional studies are still needed. V1396M impacts the S5 subunit which might disrupt the interaction of the alpha 1 subunit with the other $Ca_v2.1$ subunits to form a functional channel. When this variant was expressed in cells, the amount of current generated by the mutant channels was higher than normal, meaning more how much Ca^{2+} was entering neurons. The hyperpolarizing shift in activation seen in cells agrees with early and easier activation of the channel, and suggests a GOF effect. That same study also looked at three other *CACNA1A* variants associated with Lennox-Gastaut epileptic encephalopathy (seizures linked to cognitive decline). **A715T** is located in S6 of the repeated domain II and was shown to have a slight GOF effect. **G232V** (S5 of domain I) and **I1357S** (S4 of domain II) seemed to have LOF effects by decreasing the expression of $Ca_v2.1$ at the cell membranes.

E1775G patients showed ataxia and cognitive impairment. This variant changes one of the highly conserved (found in all organisms) glutamate amino acids in the channel. Conserved amino acids are usually of high importance. These glutamates act as the selectivity filter that only allows Ca^{2+} in. Based on previous work done on a similar variant E1761K (another conserved glutamate) in *Xenopus* (frog) oocytes, it was suggested that E1775G might also change the channel's selectivity for Ca^{2+}, allowing other positively charged ions in, and impacting the Ca^{2+} dependent release of neurotransmitters.

R1673P was found in a patient who showed congenital ataxia, hypotonia, cerebellar atrophy, and global developmental delays. This variant is located in the S4 voltage sensing subunit and was studied in *Drosophila melanogaster* (fruit flies). When the mutant channel was expressed in flies that lacked wild-type (normal) channels, it was able to partially rescue or restore some of the phenotypes, indicating a GOF (overactive channel) variant. However, this restoration disappeared after a month, suggesting there was also neurodegeneration. Experiments in cells suggested that this variant increased activation of the Ca^{2+} channels, but that did not explain the neurodegeneration seen in the flies.

The authors concluded their review by recommending the use of *Danio rerio* (zebrafish) as an *in vivo* (whole organism) system to study *CACNA1A* mutations. They emphasized that the

physiological activities of zebrafish are more similar to humans than fruit flies are. Zebrafish have two different genes that encode for the $Ca_v2.1$ channel. Futhermore, mutations in each gene have already been generated. The *tb204a* mutant (Y1662N) and *fakir* mutant (L356V) are both loss-of-function mutants that showed reduced mobility in zebrafish larvae. Both also showed depolarizing shifts in channel activity, meaning it was harder to activate the channels. Reduced amplitude (or current) through the channels also indicated less Ca^{2+} going through. Both mutations also occur in the S6 subunit of a repeated domain, which are important for forming the actual pore. Similar mutations that are linked to FHM1 or EA2 in humans, still require in depth functional studies that aren't feasible in people. However, the viability of known zebrafish mutants and the imaging capability in zebrafish to see actual Ca^{2+} movement into neurons present a more feasible way to further assess the molecular mechanisms of disease variants. Furthermore, specific pathogenic mutations in humans, like those reviewed in this paper, can be generated in zebrafish using CRISPR technology. The authors also proposed using zebrafish as a system to screen compounds for potential new drug therapies to treat *CACNA1A*-related disorders. They based this on a previous screen done with zebrafish that successfully found new therapeutic compounds for Dravet syndrome, another channelopathy (channel disease).

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