Complex effects on $Ca_V2.1$ channel gating caused by a CACNA1A variant associated with a severe neurodevelopmental disorder

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Abstract

Ca^{2+} flux into nerve terminals via $Ca_V2.1$ channels is essential for active neurotransmitter release at neuromuscular junctions and many central synapses. Mutations in *CACNA1A*, the gene encoding the principal $Ca_V2.1$ α_{1A} subunit, cause a broad spectrum of neurological disorders. Typically, gain-of-function (GOF) mutations are associated with migraine and epilepsy while loss-of-function (LOF) mutations are causative for episodic and congenital ataxias. However, a cluster of severe $Ca_V2.1$ channelopathies have overlapping presentations which suggests that channel dysfunction in these disorders cannot always be defined bimodally as GOF or LOF. In particular, the R1667P mutation causes focal seizures, generalized hypotonia, dysarthria, congenital ataxia and, in one case, cerebral edema leading ultimately to death. Here, we demonstrate that the R1667P mutation causes both channel GOF (hyperpolarizing voltage-dependence of activation, slowed deactivation) and LOF (slowed activation kinetics) when expressed heterologously in tsA-201 cells. We also observed a substantial reduction in Ca^{2+} current density in this heterologous system. In summary, our findings indicate a complex functional effect of R1667P and support the idea that pathological missense mutations in $Ca_V2.1$ may not represent exclusively GOF or LOF.

Abridged Methods

Modeling. The AlphFold2 model of human $Ca_V2.1$ can be accessed at the following web address: https://alphafold.ebi.ac.uk/entry/O00555. Homology models of full length $Ca_V2.1$ α_{1A} subunits were generated using Phyre2 employing $Ca_V2.2$ α_{1B} as a template. The sequence of $Ca_V2.1$ α_{1A} was downloaded from the universal protein resource (Uniprot; entry: O00555). The optimal template for homology modelling was identified using the BLASTp program. The 3D structure of $Ca_V2.2$ α_{1B} (Uniprot ID: Q00975) was downloaded from PDB (PDB ID:7miy) and served as the template structure. The secondary structure of $Ca_V2.1$ α_{1A} was predicted using Phyre2. Missense mutation analysis was performed using Missense 3D. Structures were visualized in PyMol.

tsA-201 cell culture and transfection: Cells were transfected using Lipofectamine 2000. The transfection mixture contained plasmids encoding fluorescent protein-fused, The transfection mixture contained plasmids encoding fluorescent protein-fused, wild-type or mutant, human (Δ10A (−V + G), 16*17*, Δ17A (−VEA), −31* (−NP), 37a (EFa), 43*44*, and Δ47) GFP-$Ca_V2.1$, or GFP-$Ca_V2.1$ R1667P, rabbit β_4 and $\alpha_2\delta$-1 subunits (1 mg of each cDNA/well). Cells were maintained at 30°C for 24hr before electrophysiology experiments.

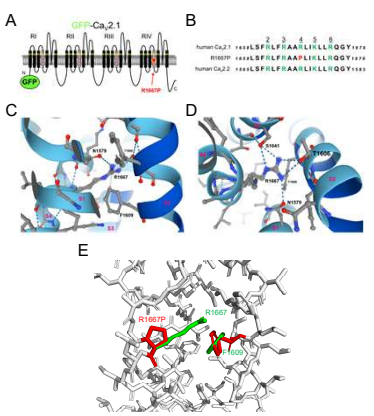
Whole-cell electrophysiology: All electrophysiological experiments were performed at room temperature (21-25ºC). Borosilicate pipettes (2.5-4.0 MW) were filled with an internal solution containing (mM): 140 Cs-Aspartate, 10 Cs_2-EGTA, 5 $MgCl_2$ and 10 HEPES, pH 7.4 with CsOH. The external solution contained (mM): 145 tetraethylammonium-Cl, 2 $CaCl_2$, 10 HEPES, 10 glucose and pH 7.4 with tetraethylammonium-OH. The mean value of C_m was 23.0 ± 0.8 pF (n = 36). Conductance-voltage (G-V) relationships were obtained from the I_{tail}-V data where individual tail current amplitudes evoked from a given test potential were normalized by the maximal tail current amplitude produced by repolarization to -40 mV. Activation, deactivation and steady state inactivation recordings were fit with a single exponential.

Statistics: Data are presented as mean ±SEM. Comparisons were by unpaired, two-tailed *t*-test unless otherwise noted; $p < 0.05$ was considered significant.

Clinical Significance:

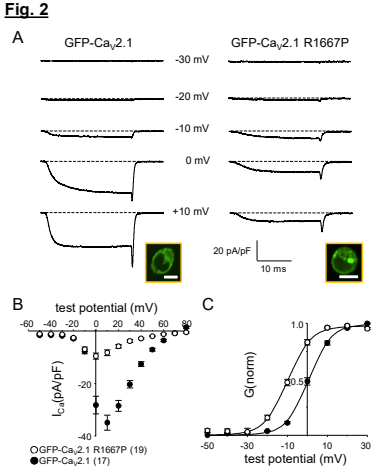
- **Humans with $Ca_V2.1$ (CACNA1A) mutations have 3 main presentations based on location of mutation**
  - **Familial hemiplegic migraine**
  - **Episodic ataxia**
  - **Epilepsy**
- **Inability to therapeutically target mutant calcium channel frequently leads to shortened life expectancy for individuals carrying $Ca_V2.1$ mutations.**

Fig. 1



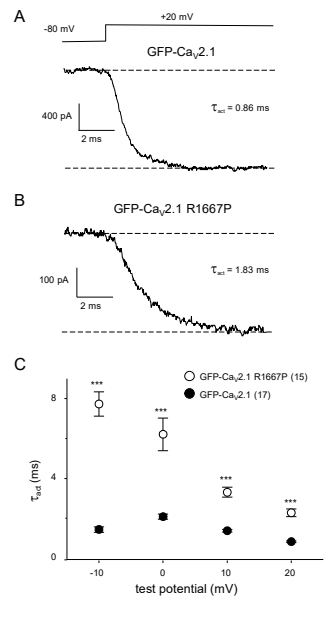
The GFP-$Ca_V2.1$ R1667P mutation is in the R4 position of Repeat IV S4 voltage sensor. The R1667 arginine is predicted to create multiple hydrogen bonds within the helix which are disrupted with the proline mutation. The lack of this bonding may change the helical structure at this site, serving as the mechanism for changes in channel function described here.

Fig. 2



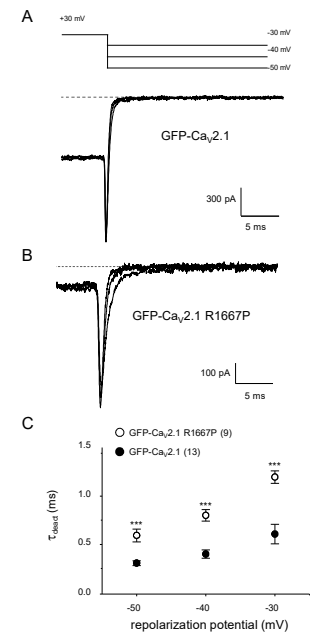
GFP-$Ca_V2.1$ R1667P mutation exhibits decreased current density and ~10mV hyperpolarizing shift in activation.

Fig. 3



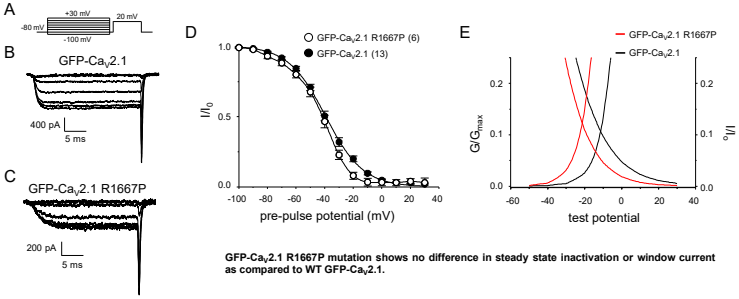
GFP-$Ca_V2.1$ R1667P mutation shows significantly slower activation kinetics (p<.001) at all potentials measured as compared to WT GFP-$Ca_V2.1$.

Fig. 4



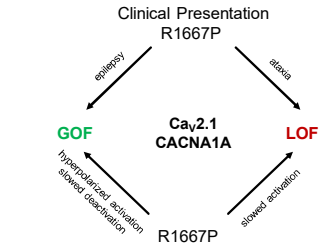
GFP-$Ca_V2.1$ R1667P mutation shows significantly slower deactivation kinetics (p<.001) at all potentials measured as compared to WT GFP-$Ca_V2.1$.

Fig. 5



GFP-$Ca_V2.1$ R1667P mutation shows no difference in steady state inactivation or window current as compared to WT GFP-$Ca_V2.1$.

Clinical and Biophysical R1667P Correlation



Clinical presentation taken from: Gauquelin L, Hawkins C, Tam EWY, Miller SP, Yoon G, Pearls & Oy-sters: Fatal brain edema is a rare complication of severe CACNA1A-related disorder. Neurology. 2020 Apr 7;94(14):631-634.

Preliminary conclusions

1. **R1667P sits in the S4 voltage sensor of Repeat IV of $Ca_V2.1$ and is hypothesized to disrupt hydrogen bonding within the helix**
2. **R1667P mutation reduces current density and has a ~10 mV hyperpolarizing activation**
3. **R1667P induces significantly slower activation and deactivation kinetics while having no clear effect on closed state inactivation**

Together these data support a mixed GOF and LOF phenotype for the R1667P mutation. This complex characterization reflects the symptoms exhibited by the patients known to carry the 1667P mutation; they also present with both GOF and LOF symptoms.

Future directions

1. Study R1667P in a knock in *Danio rerio* fish model. Specifically probe effects of 1667P on synaptic function and locomotive behavior.
2. Characterize other point mutations in CACNA1A identified from patient samples.
3. Drug screen for each $Ca_V2.1$ mutation to identify drug that will most closely normalize channel function to that of WT $Ca_V2.1$. This information will be informative to scientists and physicians.

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