For presentation at the American Epilepsy Society (AES) Annual Meeting December 2-6, 2022
Nashville, Tennessee; https://www.aesnet.org/AES-annual-meeting

Title: Targeted therapy corrects cellular dysfunction, ataxia, and seizure susceptibility in a mouse model of a Progressive Myoclonus Epilepsy

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Rationale: A recurrent heterozygous variant c.959G>A (p.Arg320His) in *KCNC1* causes progressive myoclonus epilepsy type 7 (EPM7), characterized by epilepsy, myoclonus, and ataxia. *KCNC1* encodes the voltage-gated potassium (K+) channel subunit Kv3.1; Kv3.1-containing channels are expressed in defined subsets of brain neurons that fire at high frequency as determined by the unique biophysical properties of Kv3 channels. We hypothesize that complex features of EPM7 are due to dysfunction of Kv3.1-expressing neurons in distributed brain regions. To investigate disease mechanisms and to develop and validate novel therapeutic strategies, we generated an experimental mouse model – *Kcnc1*-p.Arg320His/+ (H/+) mice – via CRISPR/Cas9, which harbor the same causal variant as that found in human disease.

Methods: We used whole-cell patch clamp to record the intrinsic electrophysiological properties of Kv3.1-expressing parvalbumin-positive cerebral cortex GABAergic interneurons (PV-INs) and cerebellar granule cells (CGCs) in acute brain slices prepared from H/+ vs. age-matched wild-type littermate controls at various time points (P18-21 and 2, 4, and 6 months). Cerebral cortex hyperexcitability was assessed via sensitivity to audiogenic seizures and pentylenetetrazol (PTZ) kindling; motor function was assessed via hindlimb clasping, accelerated Rotarod, and elevated beam walking assays in male and female H/+ and age-matched wild-type littermate controls at various time points. We tested the effect of augmenting Kv3 current with a novel Kv3 channel modulator AUT00206 (AUT6; Autifony Therapeutics Limited) at various concentrations *in vitro* and *in vivo*.

Results: H/+ mice demonstrated age-dependent motor abnormalities in all behavioral tests, and exhibit a heightened susceptibility to seizure stimuli as well as age-dependent spontaneous seizures. Kv3.1-expressing CGCs and neocortical PV-INs displayed abnormal firing parameters consistent with Kv3 channel dysfunction, including decreased firing frequency, action potential broadening, and spike irregularity. There was abnormal short-term synaptic plasticity at PV-IN:pyramidal cell and CGC:Purkinje cell connections. CGC abnormalities were corrected with application of 30 or 100 µM AUT6. A single intraperitoneal injection of 30 mg/kg AUT6 *in vivo* improved performance of H/+ mice on the Rotarod test and protected mice from PTZ-induced kindling.

Conclusions: *Kcnc1*-p.Arg320H/+ mice exhibit seizure susceptibility and progressive ataxia, and hence represent a model with construct- and face-validity with which to study EPM7. We identified ion channel, cellular, and synaptic dysfunction of Kv3-expressing neurons throughout the brain. Deficits could be reversed with the novel Kv3-channel modulator AUT6, which demonstrates therapeutic potential for the treatment of human patients with EPM7.

Sources of Funding: National Ataxia Foundation Postdoctoral Fellowship Award (H.F.); The Children's Hospital of Philadelphia IDDRC New Program Development Award via NIH NINDS P50 HD105354; NIH NINDS NS122887 (E.M.G.); Autifony Therapeutics, Limted. (E.M.G.)