Gain of function of *KCNH1* induces hypoexcitability in cortical excitatory neurons derived from human induced pluripotent stem cells

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INTRODUCTION

KCNH1 encodes Kv10.1 (ether-à-go-go, Eag1), the founding member of the EAG family of voltage-gated potassium (Kv) channels. The gene was first identified and cloned in *Drosophila*. Mutant flies exhibit leg-shaking behavior upon ether treatment. *KCNH1* knockout (KO) mouse and zebrafish models have been generated. While KO zebrafish have developmental deficits and die in the embryonic stage, *KCNH1* KO mice are largely normal. In 2015, missense mutations in *KCNH1* were found in patients with Temple-Baraister syndrome (TBS) and Zimmermann-Laband syndrome (ZLS). Patients predominantly have seizures, intellectual disability, and other developmental deficits, including a lack of nails from the thumb and great toes. Till now, about 50 cases and 40 unique mutations have been reported. Notably, most of the disease-associated mutations are gain-of-function (GOF) mutations. While the gene has been well studied in animal models, it has not been studied in human neurons, especially in the context of human diseases.

APPROACH

Using CRISPR/Cas9, we introduced one recurrent GOF mutation, I494V, to a reference human induced pluripotent stem cell (iPSC) line. Two independent mutant clones carrying I494V and one control clone that underwent the CRISPR process but did not have the mutation were used in the study. After finishing the extensive quality control experiments, we differentiated the mutant and control iPSCs into cortical glutamatergic neurons (iGlut neurons) and performed molecular and functional studies. We examined the effect of I494V on gene expression at transcriptional and translational levels using RT-qPCR and western blot, on protein expression pattern using immunocytochemistry, on neuronal excitability using conventional patch-clamp and population-based multielectrode array assays (Maestro MEA system, Axion BioSystems). We further designed antisense oligonucleotides (ASOs) to target *KCNH1* GOF.

CONCLUSIONS AND FUTURE STUDIES

We find that the I494V mutation leads to lower spiking and bursting activities in iGlut neurons in MEA assays. Such hypoactivities are likely attributed to altered intrinsic activities, as I494V neurons have decreased RMP in patch clamp. At the molecular level, I494V does not affect gene expression level or pattern. Notably, our preliminary data from *KCNH1* ASOs studies suggest that reducing protein levels can effectively restore the hypoactivity associated with I494V. Future studies will focus on 1) dosing *KCNH1* ASO and 2) testing the effect of the *KCNH1* ASO on other GOF mutations. The model system we established will help to understand the pathobiology of seizures associated with *KCNH1* GOF mutations and to test various treatment modalities, including ASOs.

RESULTS

Fig. 1. Generation and characterization of I494V knock-in iPSCs

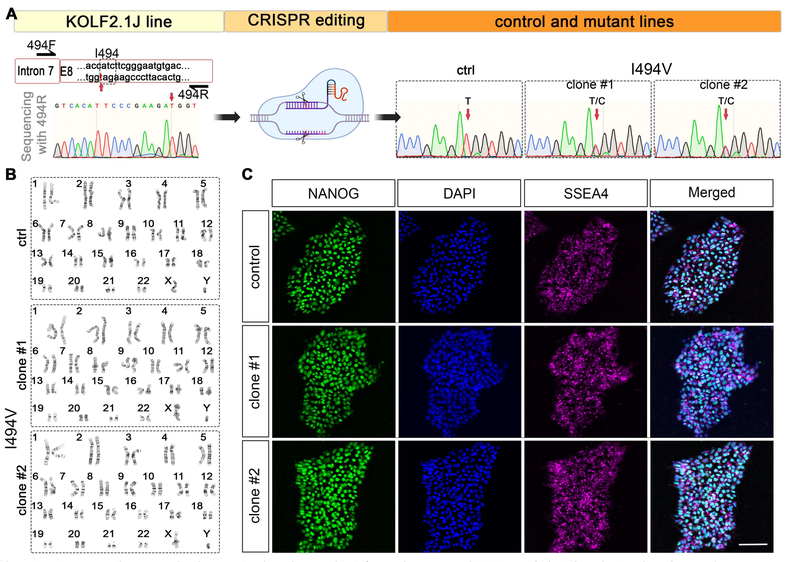

Fig. 1. Generation and characterization of ctrl and I494V iPSCs. (A) The introduction of I494V using CRISPR/Cas9. (B-C) Karyotyping and pluripotency examination of ctrl and I494V iPSCs.

Fig. 2. Differentiation of ctrl and I494V iPSCs into cortical glutamatergic neurons

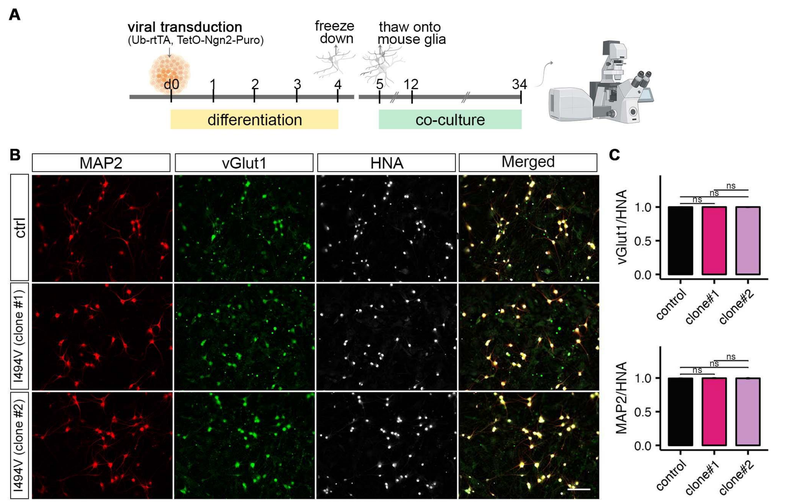

Fig. 2. Differentiation of iPSCs into iGlut neurons. (A) The differentiation protocol used in this study. (B) Immunocytochemistry (ICC) of MAP2 (neuronal marker), vGlut1 (glutamatergic neuron marker), and HNA (human nuclear antigen). n = 1022-1161 cells from 3 diffs. One-way ANOVA. ns, not significant.

Fig. 3. I494V does not affect gene expression level or pattern

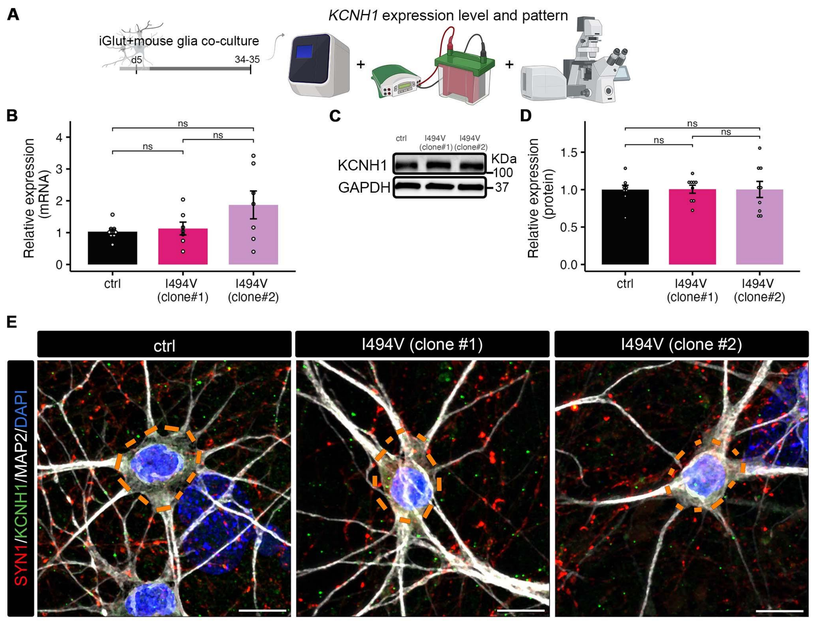

Fig.3. Molecular effect of I494V on *KCNH1* expression level and pattern. (A) A schematic presentation of the experimental flow. (B) qPCR data. n = 6-8 from 3 diffs. (C-D) Representative western blot and data quantification. n = 9 from 3 diffs. One-way ANOVA. ns, not significant. (E) Staining of MAP2 (neuronal marker), SYN1 (presynaptic marker), DAPI (nucleus marker), and KCNH1 in iGlut neurons.

Fig.4. I494V reduces RMP but does not affect AP phenotypes in patch clamp

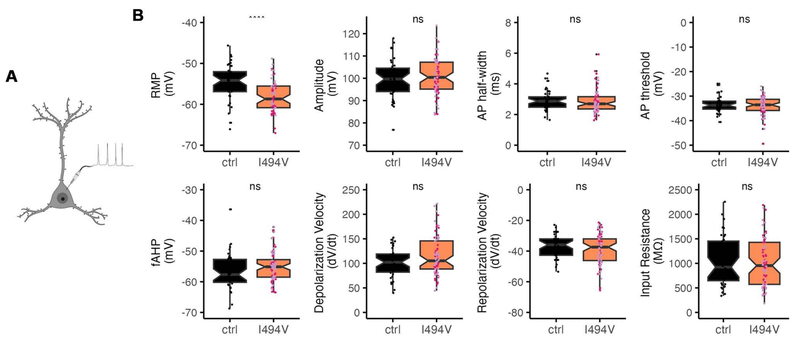

Fig. 4. Whole-cell current clamp of control and I494V iGlut neurons. (A) A schematic presentation of the patching. (B) Passive properties and action potential phenotypes of iGlut neurons. n = 33-39 from 3 diffs. unpaired *t*-test. ****p < 0.0001, ns, not significant.

Fig. 5. I494V iGlut neurons are hypoactive in MEA

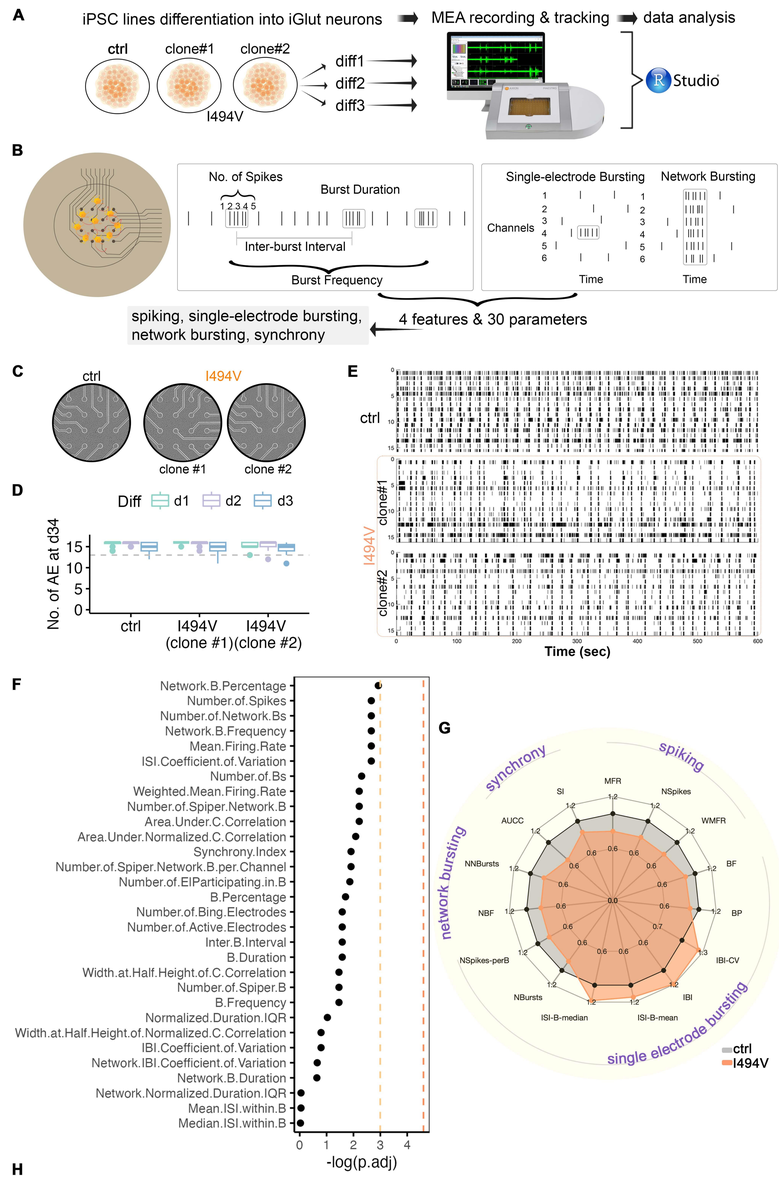

Fig. 5. Neuronal and network activities of ctrl and I494V iGlut neurons in MEA. (A) Experimental design and flow. (B) MEA well and parameter definition. (C) Representative images of MEA plating. (D) Boxplot of the number of active electrodes at d34 from three lines and three diffs. (E) Representative raster plots of MEA data from three lines. (F) Comparison of 30 parameters between two mutant lines. (G) Radar plot of significant MEA parameters across 4 features between control and mutant lines. Data were normalized. (H) Weighted mean firing rate, burst frequency, and network burst frequency between ctrl and I494V iGlut neurons. n = 65-69 per genotype. Wilcoxon test with Benjamini-Hochberg post hoc. * p < 0.05, ** p < 0.01, ****p < 0.0001.

Fig. 6. *KCNH1* ASO treatment can restore the low firing rate of I494V neurons

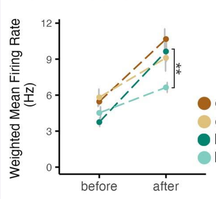

Fig. 6. Weighted Mean Firing Rate of ctrl and I494V neurons before and after *KCNH1* ASO treatment. n = 10-12 per condition. **p<0.01.

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