Development of iPSC-derived Pre-clinical Drug Screening Assays for KIF1A Associated Neurological Disorder

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Abstract

KIF1A Associated Neurological Disorder (KAND) is a rare and progressive neurodegenerative disorder caused by mutations in the KIF1A gene. Affecting over 500 diagnosed individuals, this disorder has a broad phenotypic presentation, including spastic paraplegia, seizures, hypotonia, optic nerve atrophy, cerebral and cerebellar atrophy, and intellectual disability. Despite the overwhelming need for therapeutics, there are no clinical trials, let alone approved therapies for KAND in which the lack of established and reproducible assays for therapeutic discovery and development is a major barrier. Therefore, we generated iPSC-derived glutamatergic and GABAergic neurons from E253K and P305L KIF1A mutation patients in order to develop pre-clinical assays for KAND drug discovery. Here, we describe phenotypic assays that represent the clinical manifestations of KAND i.e., developmental delay and seizures, that can be used to screen for therapeutics. KIF1A mutations lead to impairments in neurotrophic support of axonal outgrowth, cargo trafficking deficits, as well as altered neuronal electrophysiology in iPSC-derived neurons. These KIF1A mutant phenotypes further our understanding of KAND biology and represent translatable biomarkers that can be readily adapted to high-throughput screening platforms to identify KAND therapeutics.

Induced Pluripotent Stem Cells Facilitate Translational Disease Modeling for KAND Drug Discovery

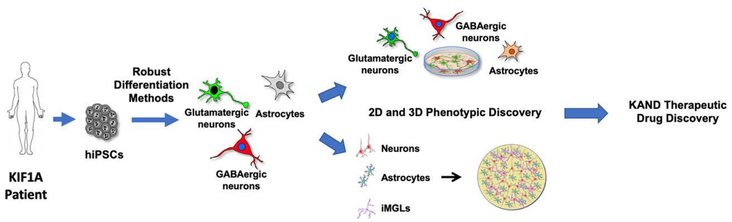


Figure 1. Schematic for the development of iPSC-derived KAND drug screening platform. KIF1A patient cells are reprogrammed to iPSCs. Robust differentiation methods developed at NeuCyte to generate neurons, astrocytes, and microglia, enable the development of 2D and 3D pre-clinical assays for KAND drug discovery.

KIF1A Targeted Therapeutics for Neurodegeneration

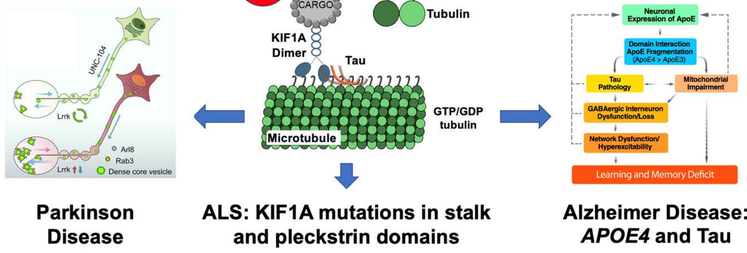


Figure 2. KAND rare disease drug discovery as a novel neurodegenerative disease target. KIF1A is a kinesin-3 motor protein implicated in other neurodegenerative diseases. KIF1A serves as a cargo receptor for huntingtin protein. KIF1A and tau both compete for binding to microtubules. KIF1A mutations in the stalk and pleckstrin domains cause ALS, and LRRK2 is a protein implicated in Parkinson Disease that acts downstream of KIF1A-mediated trafficking. Therefore, enhancing KIF1A function is a novel therapeutic target for common neurodegenerative diseases. (Some images adapted from Lessard et al., 2021, Inoshita et al., 2022, Najm et al., 2019)

iPSC-derived Glutamatergic and GABAergic KAND neurons

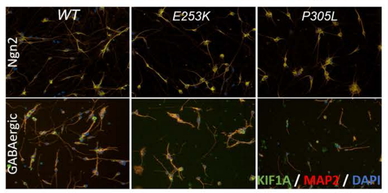


Figure 3. KAND (E253K and P305L) Glutamatergic and GABAergic neurons. Representative images of either WT, E253K, or P305L neurons (confirmed by expression of MAP2). Both neuron subtypes express KIF1A enabling examination of the effect of KIF1A mutations on neuronal function.

Development of 2D and 3D MEA-based Drug Screening Assays for KAND

	Our Rank	Passaro ranking	Top5 Passaro
Number of Bursting Electrodes	1	4	Burst percentage—Avg
Number of Spikes per Burst-Avg	2	10	Network burst percentage
Burst Duration-Avg (sec)	3	2	Number of spikes per burst—Avg
Burst Percentage-Avg	4	1	Number of bursting electrodes
Number of Spikes per Network	5	5	Number of spikes per network burst per Burst per
Channel-Avg			channel—Avg

We are concordant with 4/5 in the top 5 (Passaro). For the other top 5 parameter driving PCA ontogeny, it is 10th (Passaro) in our ranking. Best duration is our number 3 rank which ranks 13th in Passaro. Conclusion: Overall general agreement.

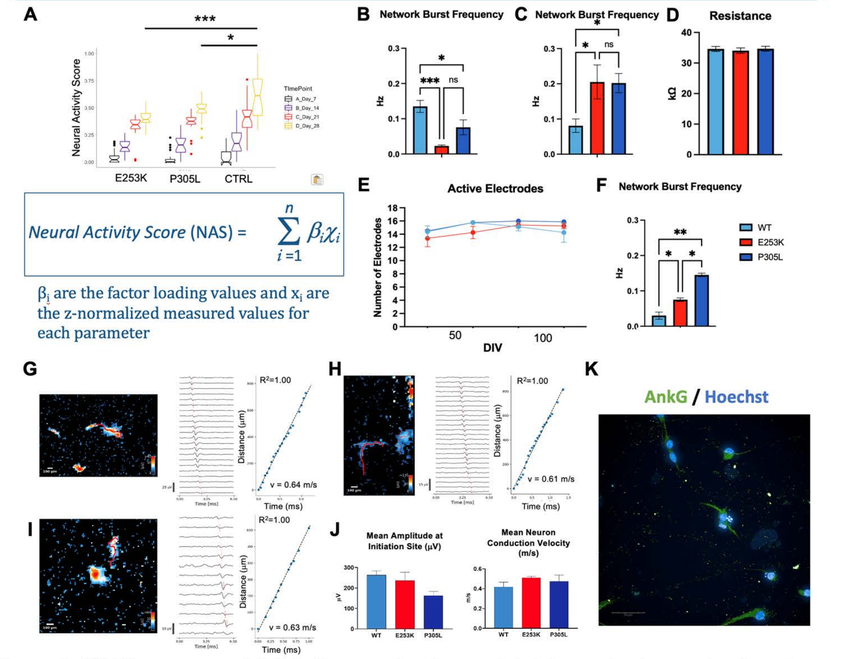


Figure 4. KAND neurons exhibit both neurodevelopmental delay and seizurgenic phenotypes. **(A)** Neural Activity Score identifies neurodevelopmental delay in KAND neurons using MEA (Axion). **(B)** Network Burst Frequency is similarly impaired also indicative of neurodevelopmental delay **(C)** KAND neurons exhibit a seizurgenic phenotype in 2D cultures only after >100d in culture. **(D,E)** Resistance and active electrodes indicate that long-term cultures are healthy **(F)** 3D cultures (<30d) accelerate detection of seizurgenic phenotypes. **(G, H, I)** Axon tracing and representative axon conduction velocity of WT and KAND mutation neurons (E253K, and P305L) in 3D cultures. **(J)** Quantification of amplitude of action potential at the Axon Initial Segment (AIS) and axon potential conduction velocity **(K)** Representative image of an image-based secondary validation of AIS impairments with AnkrinG staining.

KAND iPSC-derived Neurons Exhibit Axonal Trafficking Deficits

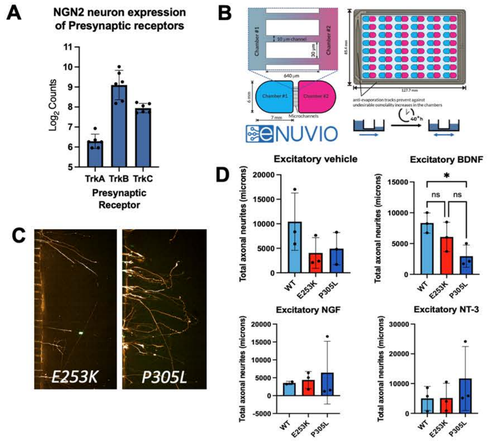


Figure 5. KAND NGN2 neurons display specific deficits in neurotrophic factor-mediated axonal outgrowth. (A) Human iPSC-derived NGN2 neurons express neurotrophic receptors by RNA-seq analysis. **(B)** Microfluidic devices enable high-throughput screening of axonal phenotypes. **(C)** Representative images showing axonal outgrowth of NGN2 neurons with KAND mutations (E253K and P305L). **(D)** KAND neurons exhibit a BDNF-specific impairment of axonal outgrowth.

KAND iPSC-derived Neurons Exhibit Neurite Deficits

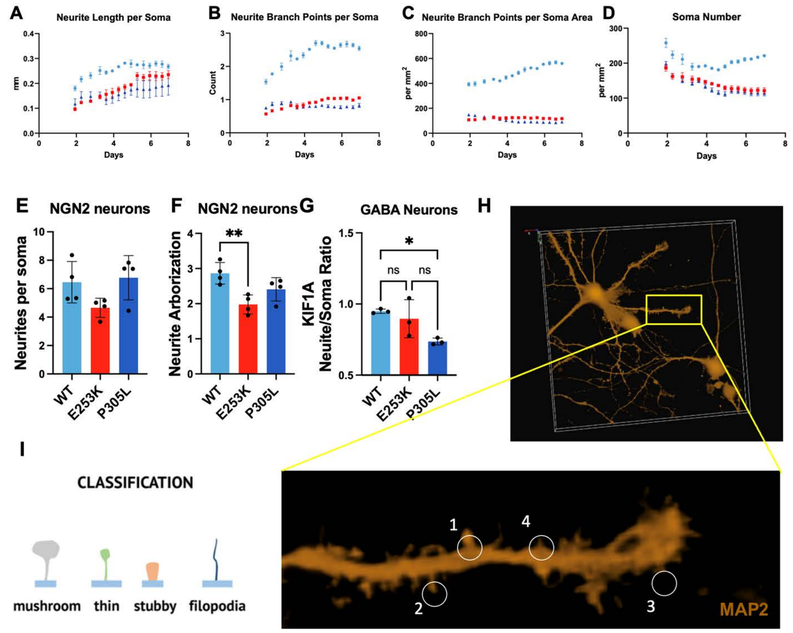
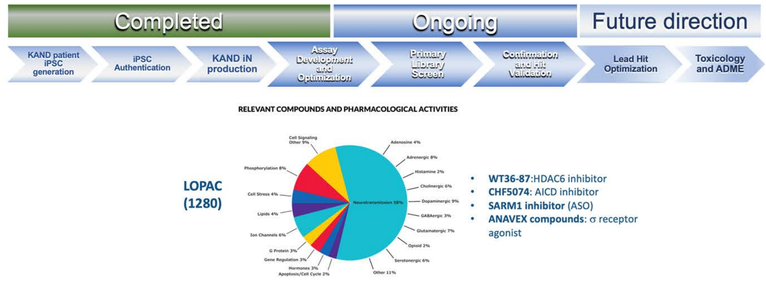


Figure 6. KAND neurons display neurite deficits. (A-D) Live cell imaging of NGN2 neuron neurite outgrowth identified deficits in neurite branching. **(E,F)** Quantification of NGN2 neurites by ICC also identifies KAND-associated defects in neurite outgrowth. **(G)** KAND GABAergic neurons exhibit impaired KIF1A neurite/soma ratios. **(H, I)** Representative immunocytochemical images of NGN2 neurons stained with MAP2 which can be used to quantify the types of dendritic processes, which can serve as a secondary validation of the neurodevelopmental delays of KAND neurons captured by MEA.

Future Directions and Conclusions



- iPSC-derived KAND neurons and co-cultures capture phenotypes that can enable high-throughput drug screening
- 3D organoid/assembloid exhibit accelerated maturation enabling screening
- Primary Drug Screening and Drug Discovery for rare diseases like KAND may uncover novel therapeutic targets for other neurodegenerative diseases
- Isogenic models may further enhance pre-clinical assay development

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