Mutation Effect, Mechanism and Drug Discovery using Epileptic Patient iPSCs

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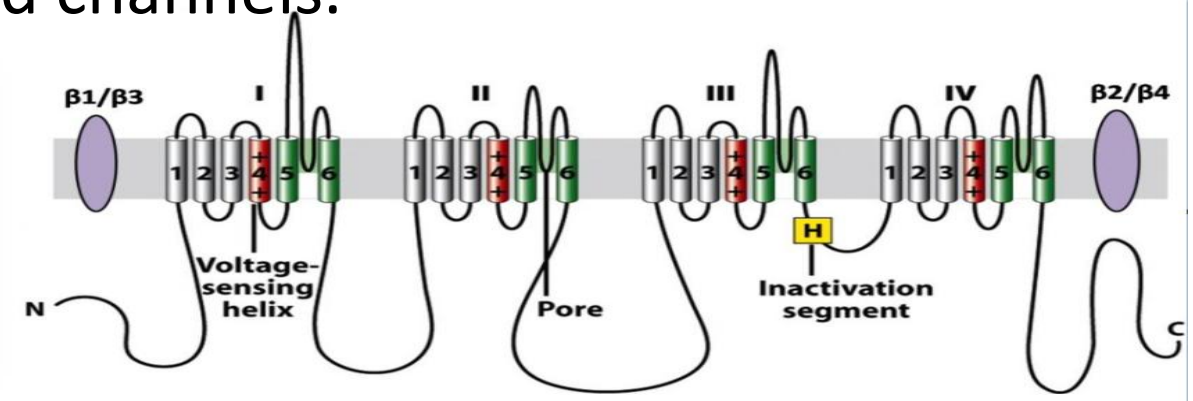
Background

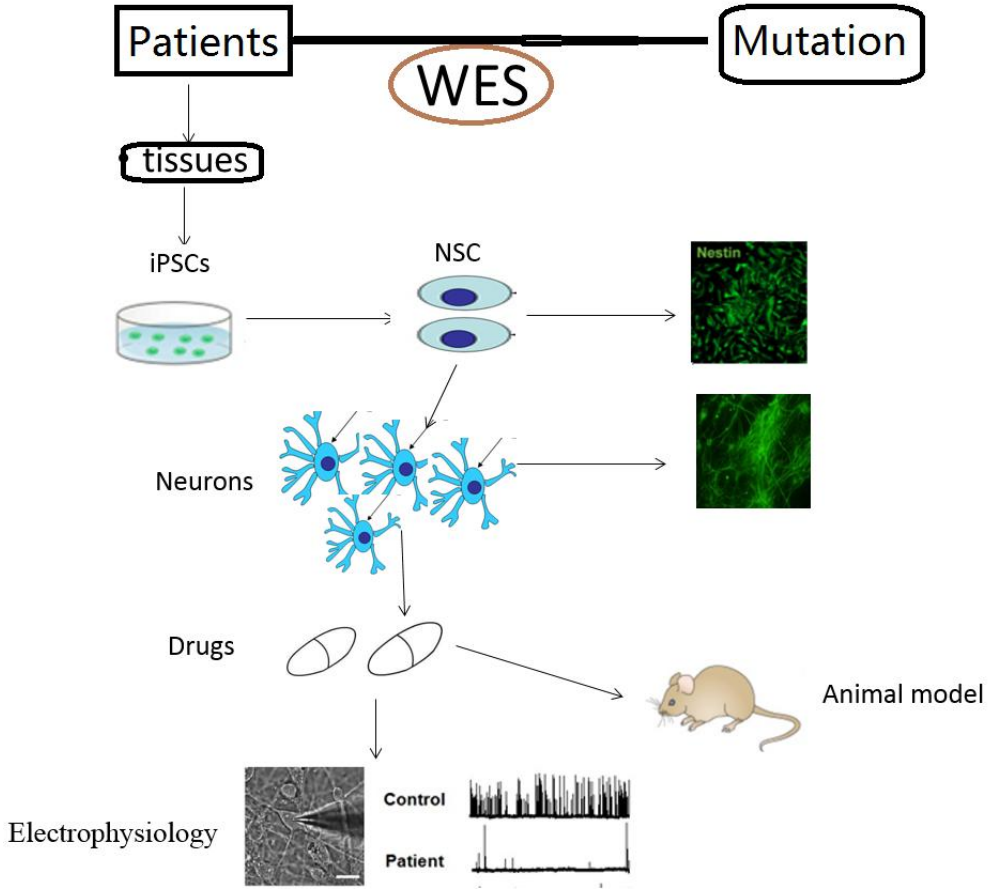
- **Epilepsy (癲痫):** a group of neurological disorders characterized by epileptic seizures that can vary from brief and nearly undetectable periods to long periods of vigorous shaking.
- **Ion Channel**: the Pathogenicity mechanism of epilepsy, including voltage gated channels [**sodium(Na**+), potassium (K+) and calcium (Ca+)] and ligand gated channels.



Method

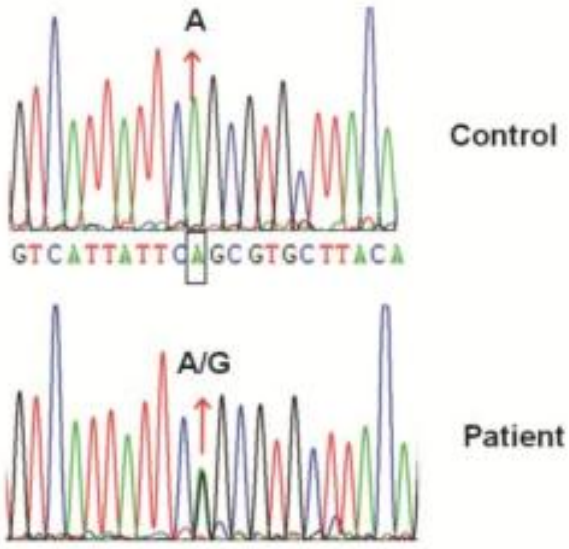
- **Epileptic patient:** a 10 year old boy with family epileptic history, noresponse to the treatment with carbamazepine etc.(30%-40%)

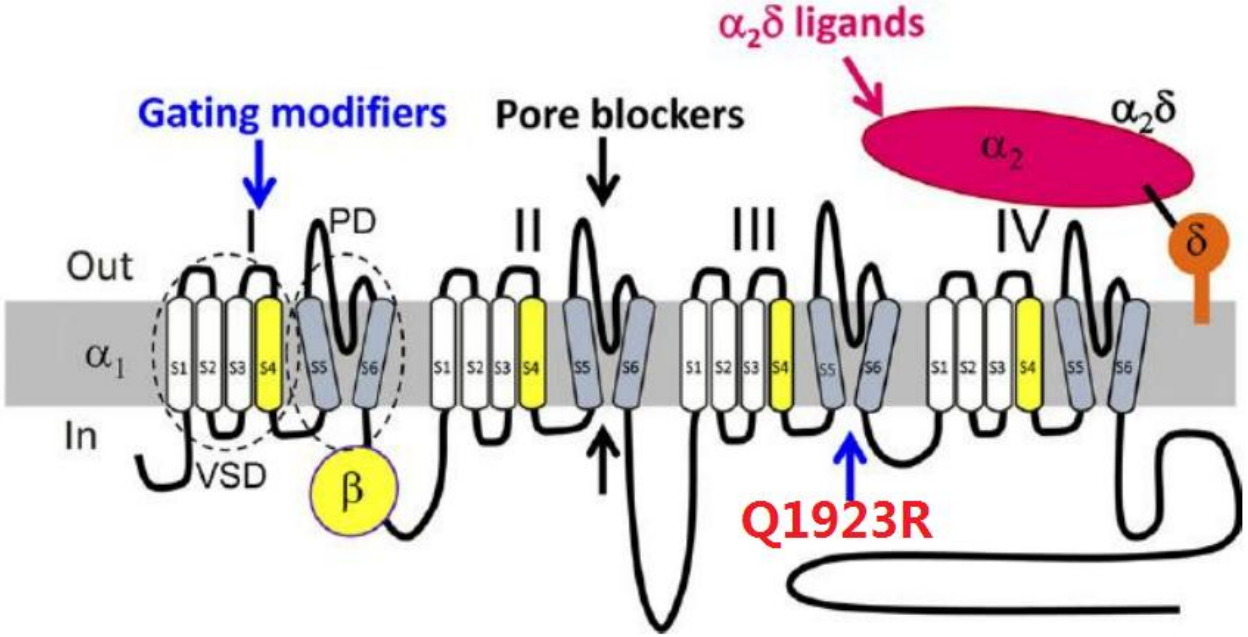
- WES
- Reprogramming
- Repairing
- Differentiating
- Electrophysiology
- Drug discovery
- Safety testing



WES (Whole exon sequencing)

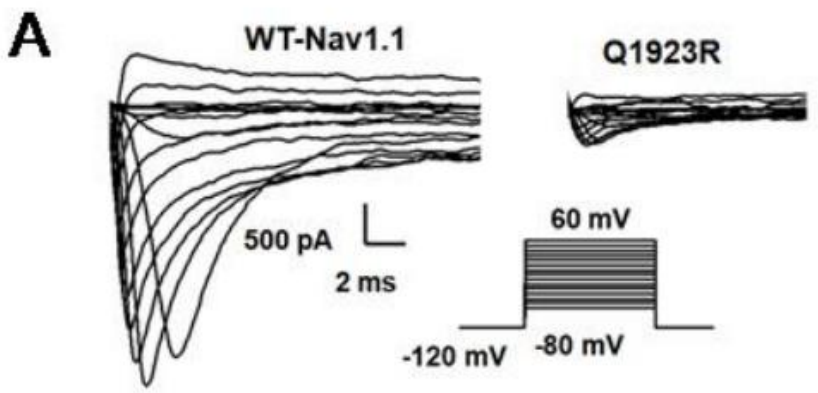
- Sequencing result: **SCN1A** (c.5768A>G)
- encoding α subunit of Nav1.1 channel

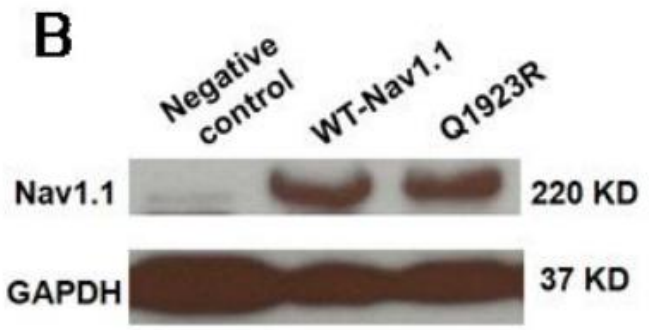




Mutation Effect Identification

- HEK293T cell model
- Transfection the plasmids of mutant \wild-type Nav1.1 α Subunit with wild-type β 1 and β 2 Subunits

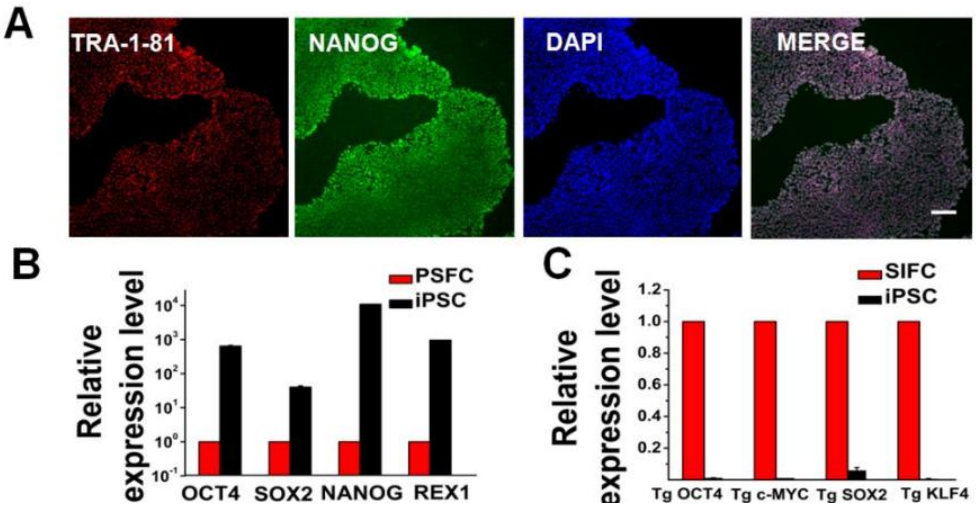




- Result: Loss of function mutation

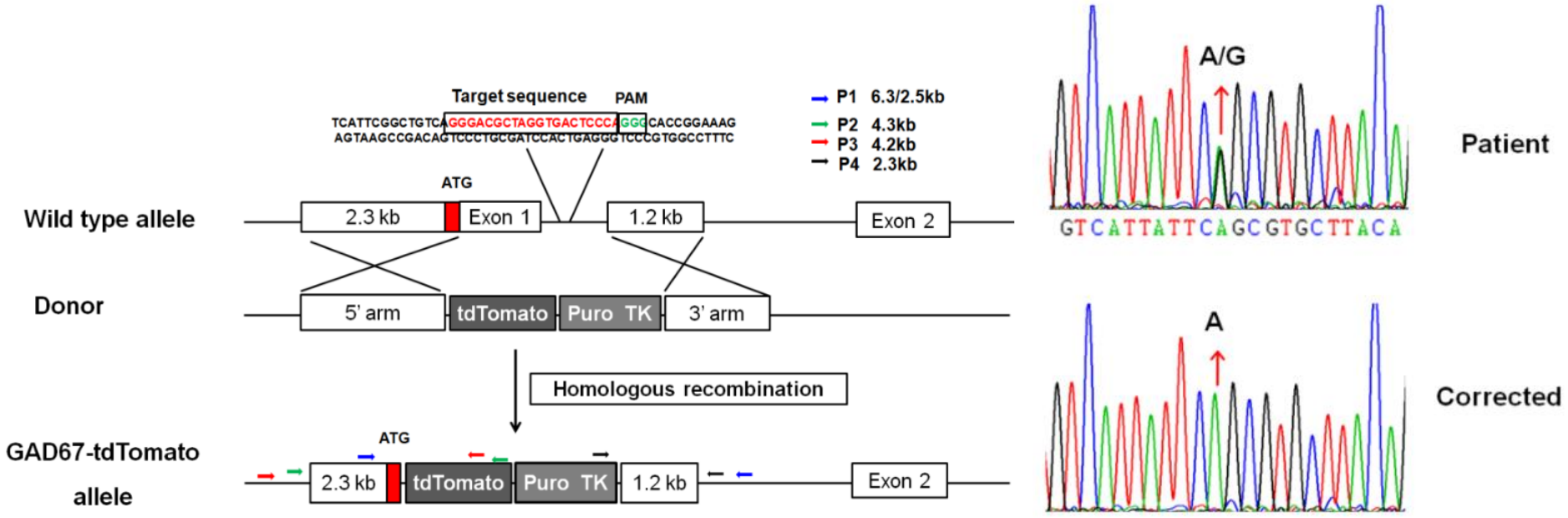
IPSc (Induced pluripotent stem cells)

- Skin tissues from the patient and normal person
- Reprogramming with cytokines Oct4，Sox2，Klf4 and c-Myc.
- Expressing markers TRA1-81 and NANOG



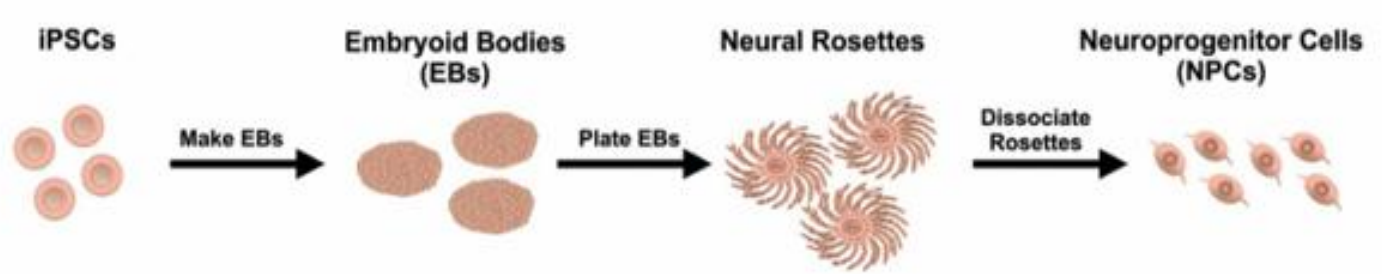
CRISPR/Cas 9 Gene Edit

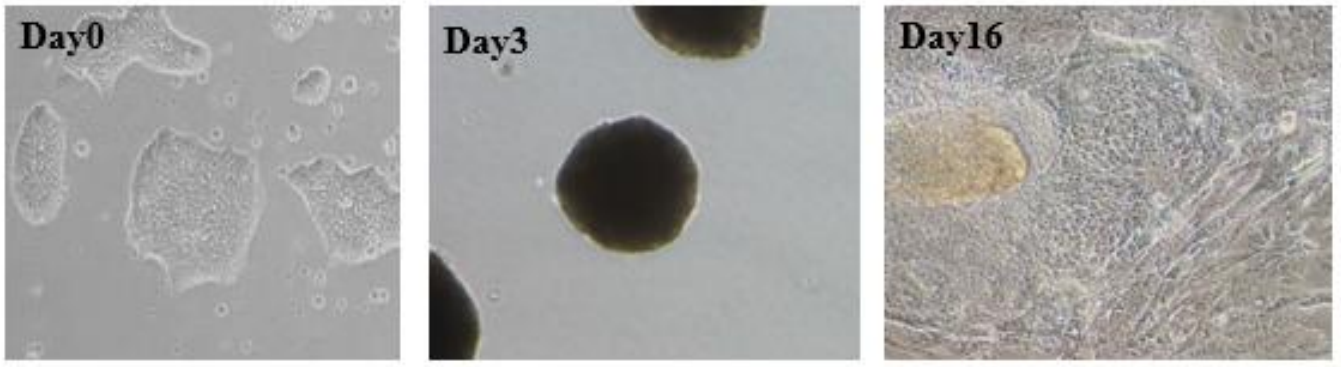
- Repairing iPS cell lines from epileptic patients by homologous recombination technology



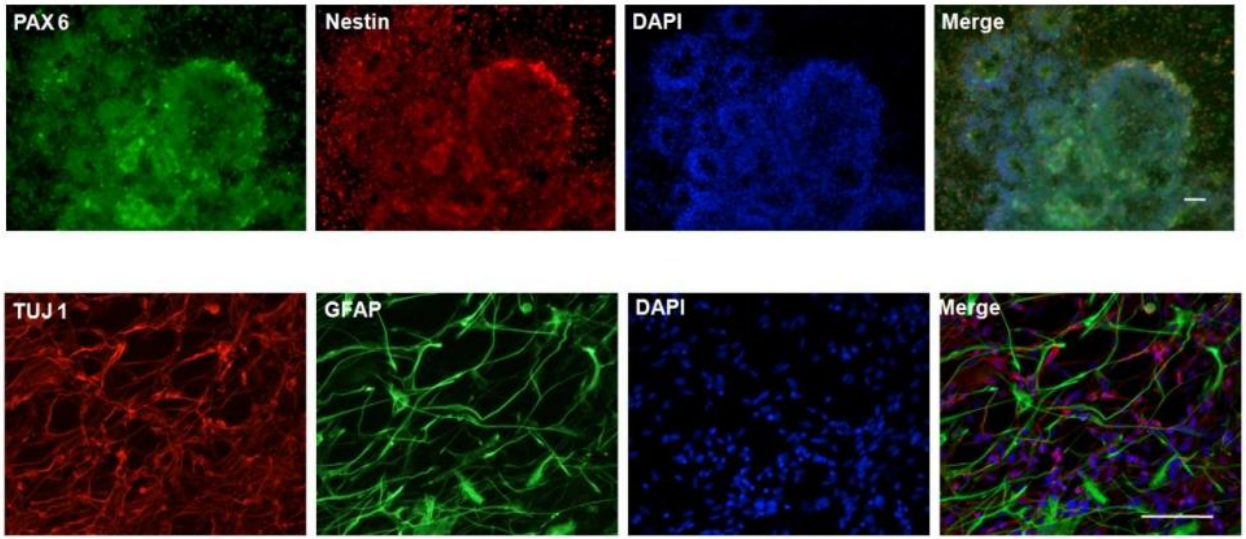
Differentiation of Neurons

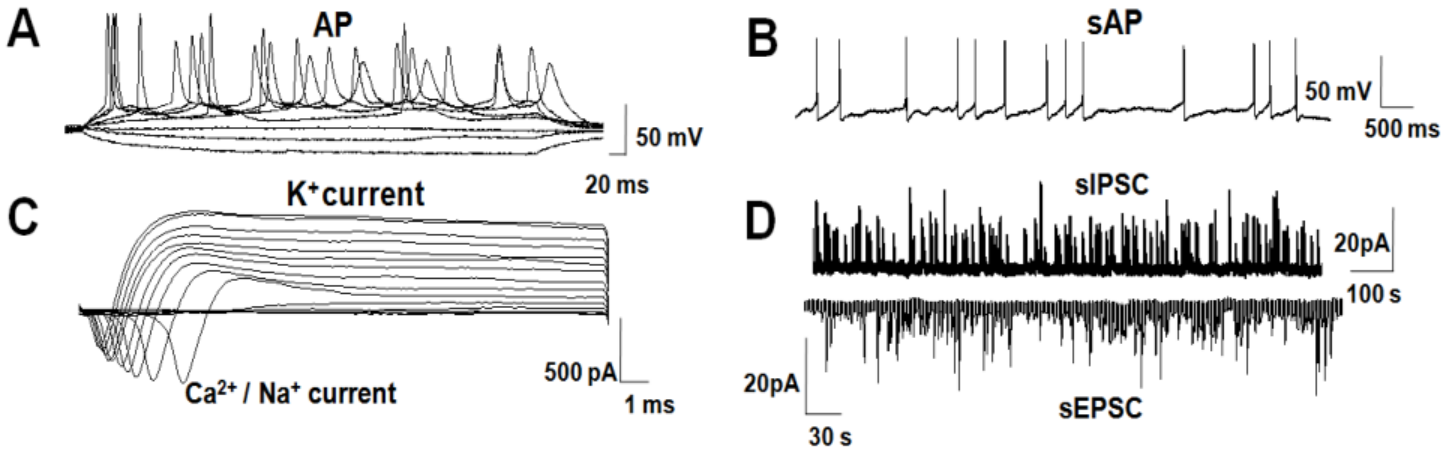
- Differentiating to excitatory glutamatergic neurons, inhibitory GABA neurons and glial cells by Classical EB ball differentiation method





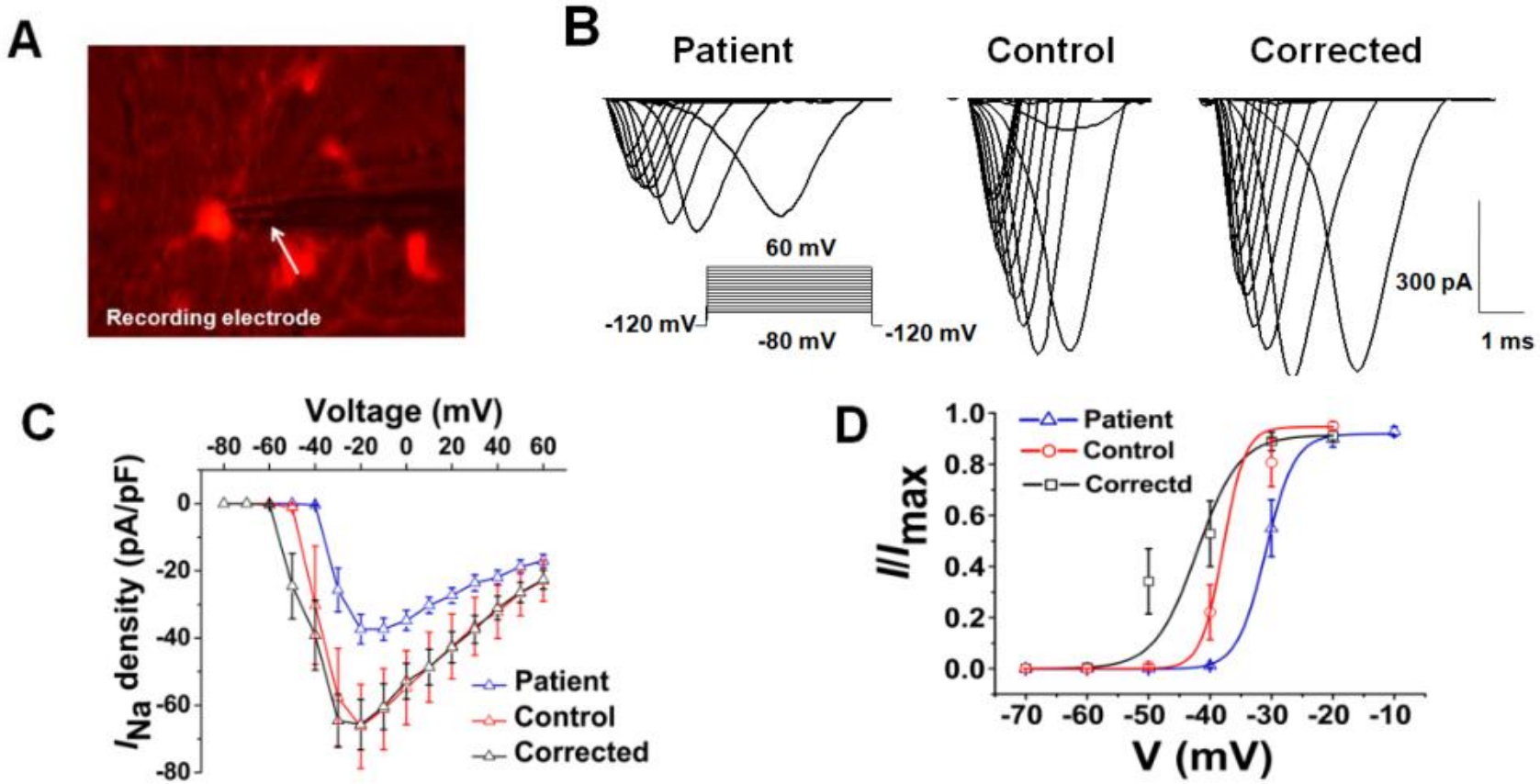
Identification

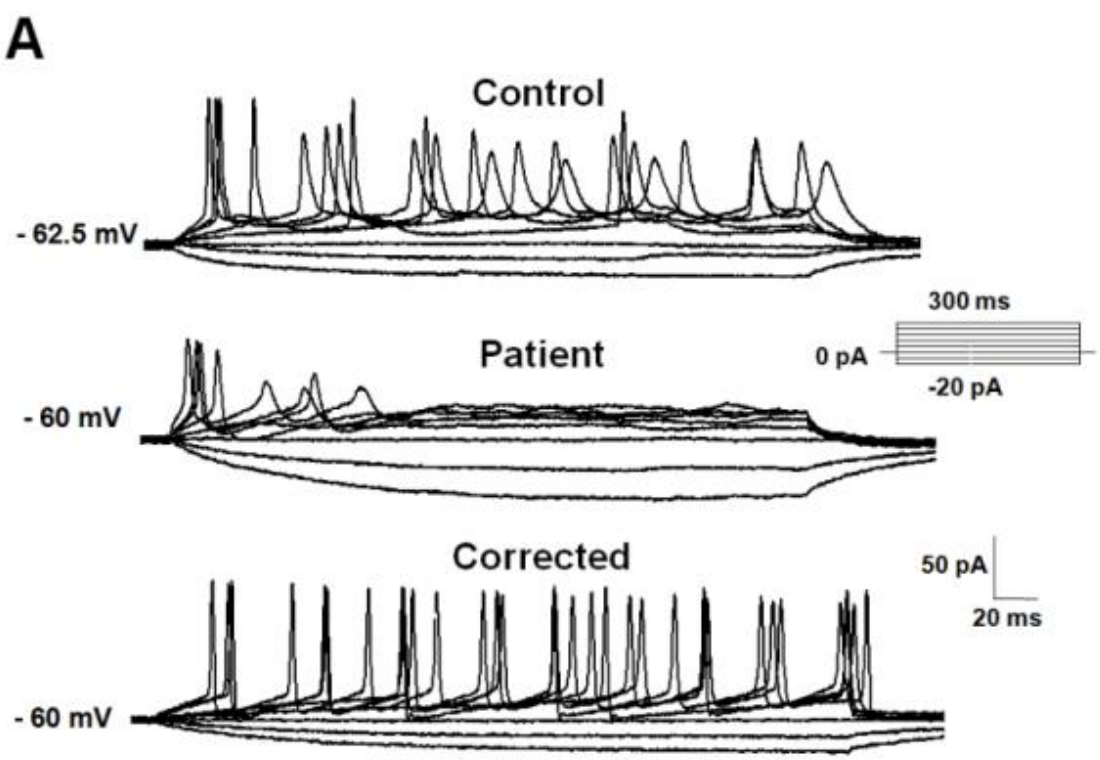


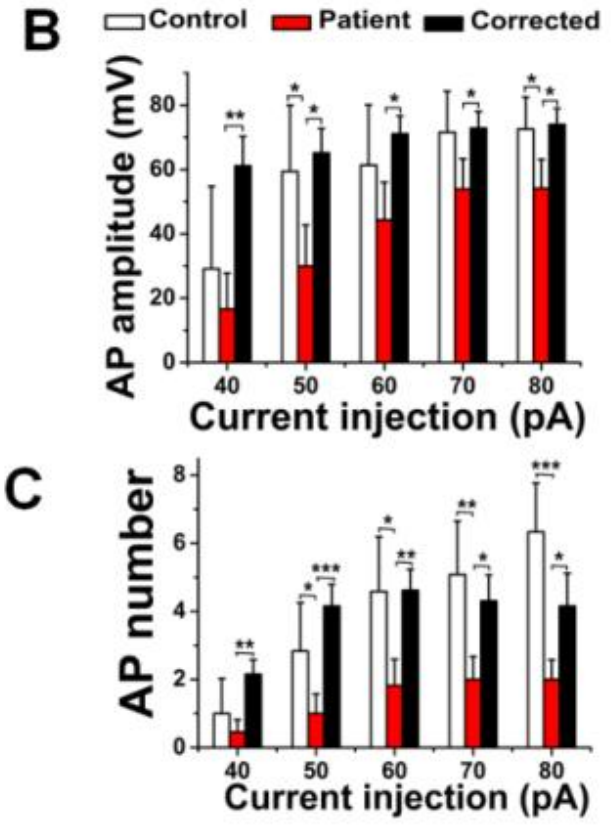


(A) evoked action potentials. (B) spontaneous action potentials.
(C) voltage gated ion channel current. (D) spontaneous postsynaptic currents.

Functional changes of Na+ channel




A
Control
- 62.5 mV
300 ms
0 pA
-20 pA
Patient
- 60 mV
Corrected
50 pA
20 ms
- 60 mV


B
Control
Patient
Corrected
AP amplitude (mV)
0
20
40
60
80
40
50
60
70
80
Current injection (pA)
C
AP number
0
2
4
6
8
40
50
60
70
80
Current injection (pA)

Drug discovery

Membrane stabilizers	• Carbamazepine,Oaxicine,Lamotrigine	**Neurons need to be pretreated with inhibitors just before record current**
Drugs of reducing neurotransmitter release	• Levyracetam	**Neurons need to be pretreated with drug or at least several hours**
Drugs that improve the stimulatory inhibitory	• Benzodiazepines,Phenobarbital,Sodium valproate,Topirate	
NMDA beta blockers	• N-methyl-d-aspartate (NMDA) receptor blocker	**Neurons need to be pretreated with inhibitors for at least 30 minutes**
Effective drugs on animal models	• Clobazam,Clemizole,Cannabidiol	**Neurons need to be pretreated with inhibitors for at least several hours**

Drug	Effect
carbamazepine	no
Zola Necita	no
topiramate	no
……	no

Compound	Effect
No.1	no
……	……
No.13	yes
No.25	yes
……	……
No.32	no

Thanks!

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