Application of induced pluripotent stem (iPS) cells in intractable childhood disorders
Lessons from Dravet synd. patient-derived iPSCs

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Summary
iPS cells invented by Prof. Shinya Yamanaka

The Nobel Prize in Medicine 2012
DRAVET syndrome
(Severe Myoclonic Epilepsy in Infancy)

- Incidence: 1 in 40,000.
- Refractory epilepsy
- Profound psychomotor delay
- Fever sensitive
- Na⁺ channel abnormalities

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**Na^+ channel (Na_v 1.1)**

Mutations found in DRAVET syndrome

Mutations found in DRAVET
All are hetero and Most are *De Novo*

Lossin C Brain & Dev 31: 114-130 (2009)

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Patient whose skin was used to establish iPS cells

- 29-year-old woman
- No perinatal abnormality
- Normal development till 6mo.
- First GTC at 7mo.
- Frequent seizures thereafter
- Non-convulsive status epilepticus

Typical DRAVET syndrome
**SCN1A mutation of the Patient**

**De novo**

c.4933 C>T  p.R1645X

**SCN1A** mutation of the Patient
Non-sense mutation (p.R1645X)

Patient iPS cells established

iPS cells Line A

iPS cells Line B

Patient fibroblast

Yamanaka’s 4 factors

Oct3/4 • Sox2 • Klf4 • c-Myc

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iPS cells remain un-differentiated

Immunostaining for iPS cells

Markers for un-differentiation

<table>
<thead>
<tr>
<th>OCT4</th>
<th>NANOG</th>
<th>Tra-1-60</th>
<th>Tra-1-81</th>
<th>SSEA4</th>
</tr>
</thead>
</table>

Marker for differentiation

SSEA1

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iPS cells show Teratoma formation

<table>
<thead>
<tr>
<th>Ectoderm</th>
<th>Mesoderm</th>
<th>Endoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal Rosettes</td>
<td>Pigmented Epithelium</td>
<td>Cartilage</td>
</tr>
<tr>
<td>Respiratory Epithelium</td>
<td>Respiratory Epithelium</td>
<td>Respiratory Epithelium</td>
</tr>
</tbody>
</table>

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iPS cells retain SCN1A mutation

Control iPS

p.R1645X (Hetero)

Patient iPS cells
Line A

Patient iPS cells
Line B

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SCN1A mRNA of Neurons

Control

Line A

Line B

Neurons derived from iPS cells

Fibroblasts

iPS cells

Embryoid body

Neurosphere

Neurons

Yamanaska’s 4 factors


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iPS cells develop to neuronal cells

βIII-tubulin neurons
GFAP glias
Hoechst 33258 nucleus

Na\textsubscript{v}1.1 Expression

50~60\% of Neurons express Na\textsubscript{v}1.1

Control (day 36)
Na\textsubscript{v}1.1 is expressed at Axon Initial Segment (AIS)

Control (day 30)
GABAergic Neurons are predominant

50~60% of Na\_v1.1 positive neurons are GABAergic

Few Glutamatergic Neurons

<1% of Na\textsubscript{v}1.1 positive neurons are Glutamatergic
Detection of $\text{Na}_v1.1$ positive neurons

Promoter Sequence

1.2kb

ATG

Venus

: 5’-Untranslated Exon

: 5’-Untranslated sequence of the first coding exon

Venus

GABA

Merge

(day 35)
Patch clamp (current clamp)
Abnormality in $\text{Na}_v1.1$ (+) neurons

Injection current

Control  DRAVET patient

Depolarization spike attenuated in inhibitory interneuron

Input-output relationship

Number of potential (>10mV) vs. Injection current (pA)

Control (n = 16)
DS Line A (n = 12)
DS Line B (n = 15)

* P < 0.05 for A
** P < 0.05 for both A and B (Wilcoxon rank-sum test)

Dysfunction of inhibitory interneuron

Relative hyper-excitation (seizures)

$\text{Na}^+$ inhibitors aggravate the unbalance

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Other iPS cells studies on Dravet syndrome
Other iPS study #1
Liu et al., Ann Neurol 2013;74:128-139

- iPS cells from two patients (IVS14+3A>T and p.Y325X)
- Pyramidal and bipolar cell like neurons are both hyper-active (excitatory)
Other iPS study #2
Jiao et al., Hum Mol Genet 201;22:4241-4252

VGLUT1/DAPI  GAD67/DAPI

iPS cells from two patients (p.Q1923R and p.F1415I)

Almost all derived neurons are glutamatergic

Glutamatergic neurons are hyper-active (excitatory)
Findings with Model mouse for Dravet syndrome
Model mouse for Dravet syndrome

Our unpublished data
**Dravet Syndrome model mouse** showed similar results to ours.

**Wild type**

**Dravet model Knock-in**

Depolarization spike attenuated in inhibitory interneuron

Scn1a knock out mice

Survival rate (%)

Postnatal days

Control
Excitatory KO
Conventional KO
Both excitatory and inhibitory KO
Inhibitory KO

The pathomechanisms of Dravet syndrome
Lessons from patient-derived iPSCs and animal models

- \( \text{Na}_v 1.1 \) channel expressed mainly in GABAergic inhibitory neurons
- Dravet syndrome mutations cause dysfunction of inhibitory interneurons
- This finding accords with clinical findings with CBZ and LTG
We thank the patient and her parents for their cooperation for the genetic study and the skin biopsy.

Electrophysiological analysis was performed by
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This study was collaborated with
Department of Physiology, Keio University School of Medicine
Prof. Hideyuki Okano

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