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# Cell-reprogramming technology and neuroscience

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Reprogramming technology enables differentiated cells of a specific cell type to be converted to another cell type with completely different functions, either through the production of induced pluripotent stem cells (iPSCs) or through direct conversion. This technology has challenged the idea that differentiated cells types are immutable entities and has enabled researchers to study the behaviour of living cell types that were previously inaccessible, such as human neurons. The study of live human neurons in a dish allows human-specific neurodevelopmental properties to be identified and the specific pathways that are defective in cells from patients with neuropsychiatric and neurodegenerative diseases to be dissected. Integrating methods to differentiate iPSCs into the relevant cell types involved in neurological disease with reproducible and scalable phenotypic assays is a new challenge for the disease-modelling field.

## Applications of iPSC-derived neural cells

*Understanding basic principles of human brain development*

iPSC-derived neurons can be used to determine the neurodevelopmental hallmarks of human neural cells (neurons and glia) and to investigate the epigenetic landscape of the cells during differentiation and the relationship between epigenetics and gene regulation. For example, given that epigenetic changes can influence gene expression and cell fate determination over time, it is highly informative to study the influence of epigenetics during human neural differentiation — something that is only possible now owing to the use of iPSC technology. These cells can also be used to study the characteristics of human iPSC-derived neural cells versus those derived from other non-human primates that are our closest relatives (such as chimpanzees). Such experiments can provide clues as to how humans evolved such a unique brain.

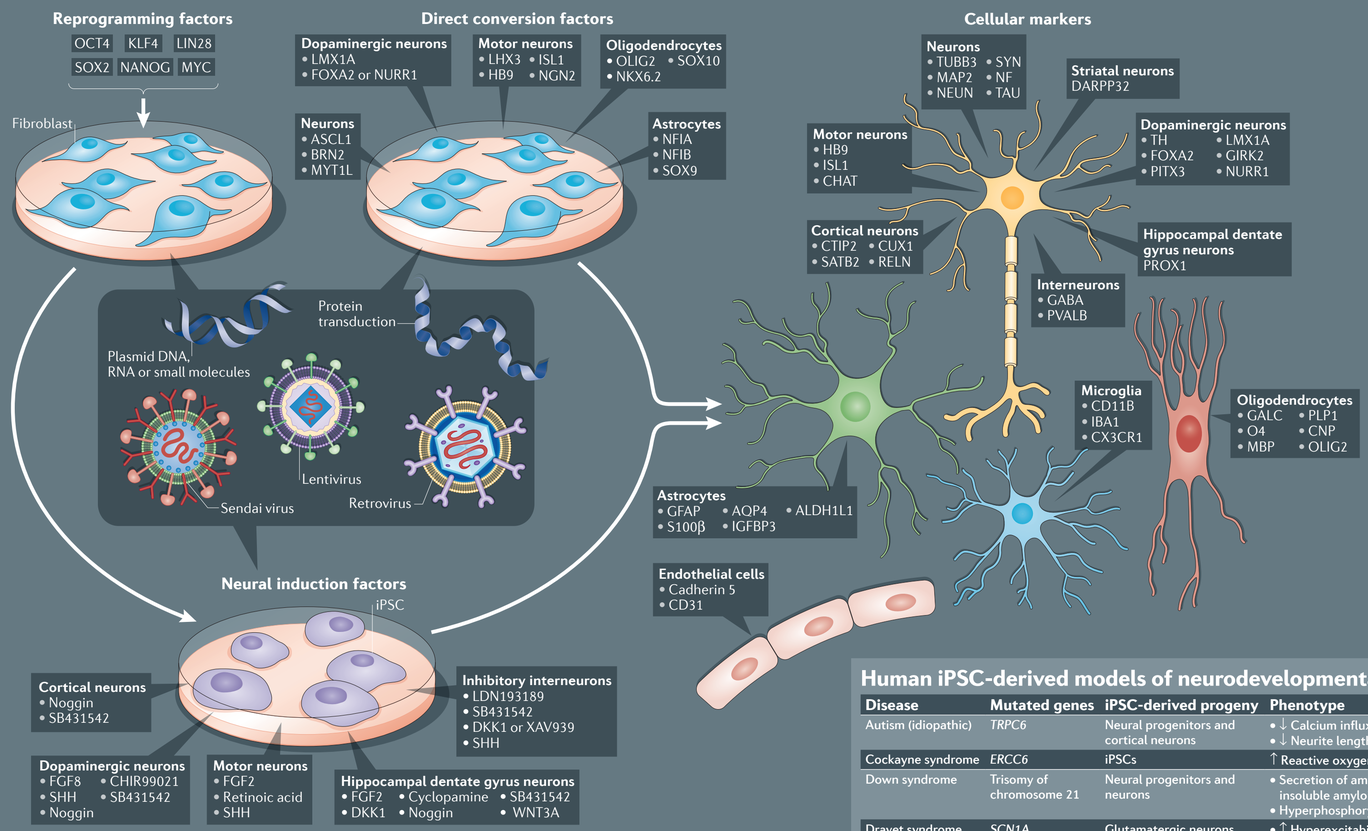
*Understanding and treating CNS disease*

Cell-reprogramming technology (see central figure) has remarkable potential to generate insights into disease mechanisms, particularly in the case of CNS diseases. Researchers can use reprogramming technology to study human disease in living neural cells that carry disease-specific genetic variants (see Tables). By comparing cells derived from patients and controls or manipulating gene expression in different neuronal subtypes using gene editing, researchers can gain an understanding of basic disease mechanisms.

Studying the development of neural cells derived from patient iPSCs will facilitate our understanding of the early steps of CNS disease processes and could therefore provide new early diagnostic tools and disease biomarkers. iPSC-derived cells can also be used in high-throughput assays for drug screening (see right figure). Indeed, reprogramming technology is already informing clinical trials. For example, iPSC-derived human neurons from patients with autism spectrum disorders that were treated with IGF1 exhibited a significantly improved phenotype *in vitro*. Modified versions of IGF1 are now in clinical trials for patients with several types of autistic spectrum disorder. It remains a challenge to predict the molecules that will work both *in vitro* and *in vivo*, but the prospect that these new models can help us to understand and potentially treat CNS diseases is exciting.

iPSC technology may also allow for the development of patient-tailored therapies: drug screening can be performed on the cells from the patient that will potentially receive the therapy, decreasing the effect of genetic background variability among individuals.

Cellular replacement therapy is also an exciting application of iPSC technology. The first patients are already receiving iPSC-derivatives via transplantation for some neurological diseases; however, caution must be taken owing to the tumorigenic potential of pluripotent stem cells.




### High-throughput phenotypic assays using iPSC-derived cells

- iPSC-derived cells from patients and control populations
- High-throughput phenotypic screen:
  - RNA sequencing and single-cell sequencing technology
  - Flow cytometry
  - Measurements of cell morphology
  - Immunohistochemistry
  - Calcium imaging
  - Multi-electrode arrays
  - Microfluidics
- Automated platform for data analysis and integration of data from different assays
- Identification of quantifiable differences in measurements between patients and controls
- Screening of drugs
- Clinical trials

## Human iPSC-derived models of neurodegenerative disorders

| Disease | Mutated genes | iPSC-derived progeny | Phenotype |
|---|---|---|---|
| Adrenoleukodystrophy | *ABCD1* | Oligodendrocytes and neurons | ↑ Levels of very-long-chain fatty acids |
| Alzheimer disease | • *PSEN1*<br>• *PSEN2*<br>• *APP* | Cortical neurons | • ↑ Amyloid-β secretion<br>• ↑ Phospho-tau (Thr 231)<br>• ↑ Active GSK3β |
| Amyotrophic lateral sclerosis | • *SOD1*<br>• *VAPB*<br>• *TARDBP* | Motor neurons and glial cells | • ↓ VAPB<br>• ↑ TDP43 |
| Familial dysautonomia | *IKBKAP* | Neural crest progenitor cells | • ↓ Neurogenesis and differentiation genes<br>• Defects in neural crest migration |
| Friedreich ataxia | *FXN* | Neurons | ↓ Frataxin protein levels |
| Hereditary spastic paraplegia | *SPG14* | Corticospinal motor neurons | • ↓ Neurite complexity<br>• ↑ Neurite swellings<br>• Impaired axonal transport |
| Huntington disease | *HTT* | Neural stem cells and astrocytes | • Susceptibility to stress<br>• Vulnerability to BDNF withdrawal<br>• ↑ Cell death<br>• ↑ Protein aggregate inclusions<br>• Altered mitochondria bioenergetics |
| Machado–Joseph disease | *ATXN3* | Glutamatergic neurons | Excitation-induced ataxin 3 aggregation |
| Parkinson disease | • *LRRK2*<br>• *PINK1*<br>• *SNCA*<br>• *PARK2*<br>• *GBA3* | Dopaminergic neurons | • Impaired mitochondrial function<br>• Sensitivity to oxidative stress<br>• ↓ Dopamine reuptake<br>• ↑ Spontaneous dopamine release<br>• ↑ α-synuclein |
| Spinal and bulbar muscular atrophy | CAG repeat in the androgen receptor gene | Motor neurons | • ↑ Aggregation of androgen receptor protein inclusions<br>• Autophagy defects |
| Spinal muscular atrophy | *SMN1* | Motor neurons | ↓ Size and number |

## Challenges and future directions

Modelling polygenic and multifactorial CNS disorders using cells derived through reprogramming requires an integrative approach that combines the capacity to detect relevant genetic determinants with the ability to search for related pathways and cellular phenotypes in reproducible and scalable bioassays. There remain several technical challenges to be overcome:

- Generating mature, adult-like neural cell types and refining the methods for detecting the maturation of neuronal activity
- Identifying genomic mutations that result in robust phenotypes in iPSC-derived cells
- Improving reproducibility and scalability of phenotypic assays
- Incorporating gene-editing technologies (such as the CRISPR–Cas9 system) to correct relevant changes in iPSCs to better understand disease mechanisms and potentially create cells for transplantation strategies
- Incorporating other CNS niche cells (for example, astrocytes, oligodendrocytes and microglia) and/or inflammatory factors into the *in vitro* model to produce more-disease-relevant phenotypes
- Incorporating the use of 3D culture models ('organoid' technology) in order to create more-realistic models of development and disease (for example, by simulating cortical layer formation)
- Developing screening platforms for new compounds that can be readily translated to *in vivo* experiments

## Human iPSC-derived models of neurodevelopmental and psychiatric disorders

| Disease | Mutated genes | iPSC-derived progeny | Phenotype |
|---|---|---|---|
| Autism (idiopathic) | *TRPC6* | Neural progenitors and cortical neurons | • ↓ Calcium influx<br>• ↓ Neurite length, complexity and spine density |
| Cockayne syndrome | *ERCC6* | iPSCs | ↑ Reactive oxygen species |
| Down syndrome | Trisomy of chromosome 21 | Neural progenitors and neurons | • Secretion of amyloid-β isoform 42 and formation of insoluble amyloid aggregates<br>• Hyperphosphorylated tau on cell bodies and dendrites |
| Dravet syndrome | *SCN1A* | Glutamatergic neurons | • ↑ Hyperexcitability<br>• ↑ Persistent sodium channel activation<br>• ↑ Evoked action potentials |
| Fragile X syndrome | *FMR1* | Forebrain neurons | Defective neurite initiation and extension |
| Phelan–McDermid syndrome | • 22q13.3 deletion<br>• *SHANK3* | Forebrain neurons | • Defects in excitatory synaptic transmission<br>• ↓ Glutamate receptor expression and number of synapses |
| Rett syndrome | • *MECP2*<br>• *CDKL5* | Neurons and neural progenitors | • Neuronal maturation defects<br>• ↓ Synapse and spine number<br>• ↓ Soma size<br>• ↑ LINE1 retrotransposition<br>• Aberrant dendritic spines |
| Schizophrenia | *DISC1* | Neurons and hippocampal dentate gyrus neurons | • ↓ Neuronal connectivity and neurite number<br>• ↓ PSD95 and glutamate receptor expression<br>• ↑ Extra-mitochondrial oxygen consumption<br>• ↑ Reactive oxygen species<br>• ↓ Spontaneous neurotransmitter release in dentate gyrus neurons |
| Timothy syndrome | *CACNA1C* | Cortical neurons | • ↓ Expression of lower cortical layer and callosal projection genes<br>• Abnormal expression of TH<br>• ↑ Production of noradrenaline and dopamine<br>• Activity-dependent dendritic retraction |



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## Supplementary information S1

### Abbreviations for the poster 'Cell-reprogramming technology and neuroscience' by Maria C. Marchetto and Fred H. Gage

ABCD1, ATP-binding cassette sub-family D member 1; ALDH1L1, aldehyde dehydrogenase family 1 member L1; APP, amyloid precursor protein; AQP4, aquaporin 4**;** ASCL1, achaete-scute homologue 1; ATXN3, ataxin 3; BDNF, brain-derived neurotrophic factor; BRN2, brain-specific homeobox 2; CACNA1C, voltage-dependent L-type calcium channel subunit-α1C; CD11B, CD11 antigen-like family member B; CD31, CD antigen CD31; CDKL5, cyclin-dependent kinase-like 5; CHAT, choline O-acetyltransferase; CNP, 2',3'-cyclic-nucleotide 3'-phosphodiesterase; CRISPR, clustered regularly interspaced short palindromic repeats; CTIP2, COUP-TF-interacting protein 2; CUX1, homeobox protein cut-like 1; CX3CR1, CX3C chemokine receptor 1; DARPP32, protein phosphatase 1 regulatory subunit 1B; DKK1, dickkopf-related protein 1; DISC1, disrupted in schizophrenia 1; ERCC6, excision repair cross-complementation group 6; FGF, fibroblast growth factor; FOXA2, forkhead box protein A2; FMR1, fragile X mental retardation 1; FXN, frataxin; GABA, γ-aminobutyric acid; GALC, galactocerebrosidase; GBA3, cytosolic β-glucosidase; GFAP, glial fibrillary acidic protein; GIRK2, G-protein-regulated inward-rectifier potassium channel 2; GSK3β, glycogen synthase kinase 3β; HB9, homeobox protein 9; HTT, huntingtin; IBA1, allograft inflammatory factor 1; IGF1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3; IKBKAP, IκB kinase complex-associated protein; ISL1, islet 1; KLF4, krueppel-like factor 4; LHX3, LIM homeobox protein 3; LIN28, protein lin-28 homologue A; LINE1, LMX1A, LIM homeobox transcription factor 1α; LRRK2, leucine-rich repeat kinase 2; MAP2, microtubule associated protein 2; MBP, myelin basic protein; MECP2, methyl-CpG-binding protein 2; MYC, Myc proto-oncogene protein; MYT1L, myelin transcription factor 1-like protein; NANOG, homeobox protein NANOG; NEUN, neuronal nuclei; NF, neurofilament; NFIA, nuclear factor 1 A-type; NFIB, nuclear factor 1 B-type; NGN2, neurogenin-2; NKX6.2,homeobox protein Nkx-6.2; NURR1, orphan nuclear receptor NURR1; O4, forkhead box protein O4; OCT4, octamer-binding protein 4; OLIG2, oligodendrocyte transcription factor 2; PARK2, parkin RBR E3 ubiquitin-protein ligase; PINK1, PTEN-induced putative kinase 1; PITX3, pituitary homeobox 3; PLP1, myelin proteolipid protein; PROX1, prospero homeobox protein 1; PSD95, postsynaptic density protein 95; PSEN1, presenilin 1; PSEN2, presenilin 2; PVALB, parvalbumin; RELN, reelin; S100β, protein S100β; SATB2, DNA-binding protein SATB2; SCN1A, sodium channel protein type 1 subunit-α; SHANK3, SH3 and multiple ankyrin repeat domains protein 3; SHH, sonic hedgehog; SMN1, survival motor neuron protein; SNCA, α-synuclein; SOD1, superoxide dismutase 1; SOX2, transcription factor SOX2; SOX9, transcription factor SOX9; SOX10, transcription factor SOX10; SPG14, spastic paraplegia 14; ST18, suppression of tumorigenicity 18 protein; SYN, synapsin; TAU, microtubule-associated protein tau; TARDBP, TAR DNA-binding protein 43 (gene); TDP43, TAR DNA-binding protein 43 (gene product); TH, tyrosine hydroxylase; TRPC6, transient receptor potential cation channel, subfamily C, member 6; TUBB3, neuron-specific class III beta-tubulin; VAPB, vesicle-associated membrane protein-associated protein B/C; WNT3A, protein Wnt3a.