Whole exome sequencing reveals 228 conserved mutations in parental and three LRRK2 Parkinson's patient-derived iPSC lines

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Introduction

Parkinson's disease is the second most common neurodegenerative disorder. Traditionally, the disease is characterized by the selective loss of dopaminergic neurons in the substantia nigra of the midbrain. Patient-specific induced pluripotent stem cells (iPSCs) provide an opportunity to model human diseases, such as Parkinson's, in relevant cell types. To provide a better research tool for studying Parkinson's disease, we generated three iPSC lines from the dermal fibroblasts of a patient with point mutations (R50H, I723V, and M2397T) in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene, the most common cause of familial Parkinson's disease. We used three different reprogramming methods: Retroviral, Sendai viral, or episomal to express *OCT3/4, SOX2, KLF4,* and *MYC* genes. These Parkinson's iPSC lines demonstrated similar cell morphology, pluripotent marker expression, and the ability to differentiate into three germ layers. Additionally, we investigated their ability to differentiate into neural progenitors.

Somatic cell reprogramming and subsequent iPSC culturing may cause random mutagenic events (references 1, 2 3). Thus, It is critical to characterize genomic integrity and stability of iPSCs for basic research and clinical application. To more effectively model Parkinson's disease and investigate the effect of reprogramming methods on mutations in iPSC lines, we sequenced exons of all 3 Parkinson's iPSC lines by whole exome sequencing. Three LRRK2 mutations were retained in 3 iPSC lines during reprogramming. Furthermore, there were 228 conserved gene mutations in all 3 iPSC lines along with the parental fibroblasts and pre-existing mutations in the parental fibroblasts accounted for approximately 70% of the missense mutations.

Methods

iPSC Generation: Dermal fibroblasts were derived from a 63 years old Caucasian male with a 2 year history of Parkinson's disease, and a 20 year history of asthma, and depression. Whole exome sequencing of the dermal fibroblasts at P5 identified 3 missense mutations in the LRRK2 gene. The donor fibroblasts at P5 were then reprogrammed to generate 3 iPSC lines (ACS-1012, ACS-1013, and ACS-1014) by Retroviral, Sendai viral, or episomal expression of *OCT3/4, SOX2, KLF4,* and *MYC* genes, respectively.

Derivation of Human Neural Progenitors: Human iPSC cell lines were expanded on Cell Basement Membrane Gel (ACS-3035)-coated dishes in Pluripotent Stem Cell SFM XF/FF medium (ACS-3002)and then cultured in N2-containing neural differentiation medium for embryoid body formation. Human iPSC-derived embryoid bodies were then seeded on poly-L-ornithine- and laminin-coated dishes for derivation of neural progenitors

Exome Sequencing: Fully characterized ACS-1012, ACS-1013, and ACS-1014 iPSC lines with normal karyotypes were passaged to P13, P12, and P16, respectively, before DNA extraction. Genomic DNA samples from 3 hiPSC lines and the parental fibroblasts at P5 were submitted to BGI (http://bgiamericas.com/) for whole exome sequencing with an Agilent's SureSelect 51 Mb array. The hg19 human genome reference sequence (http://genome.ucsc.edu/cgi-bin/hgGateway) was used to identify missense mutations in exome of the Parkinson sample.

Results

Compared to a hiPSC line-derived from a healthy subject (ACS-1007), Parkinson's iPSC lines (ACS-1012, ACS-1013, ACS-1014) showed a similar efficiency of neural differentiation into neural progenitors from iPSC-derived embryoid bodies (Figure 1). We sequenced exons of all 3 Parkinson's iPSC lines by exome sequencing and found that all 3 iPSC lines retained the parental LRRK2 gene mutations at positions 50 (R50H), 723 (I723V), and 2397 (M2397T), which have previously been reported in Parkinson's disease patients (references 4 and 5) regardless of the reprogramming methods. Furthermore, these mutations were confirmed by direct sequencing of exons 1, 18, and 49 (Figure 2). Compared to the hg19 human genome reference, the parental fibroblast and all 3 iPSC lines had over 300 genes with missense mutations and there were 228 genes with missense mutations conserved among all 4 samples (Table 1). Pre-existing mutations in the parental fibroblasts accounted for approximately 70% of the missense mutations in 3 iPSC lines and integrating (retroviral) or non-integrating (Sendai virus and episomal) reprogramming methods had a similar mutation rate in hiPSC lines. Among 228 conserved gene mutations, we identified mutations in genes associated with other known diseases by using the genecards program (http://www.genecards.org/, Table 2). We also identified nine gene mutations involved in Huntington's disease pathway by using the Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov/, Figure 3) in all 3 iPSC lines.

Figure 1. Derivation of neural progenitors from hiPSC-derived embryoid bodies (10×)

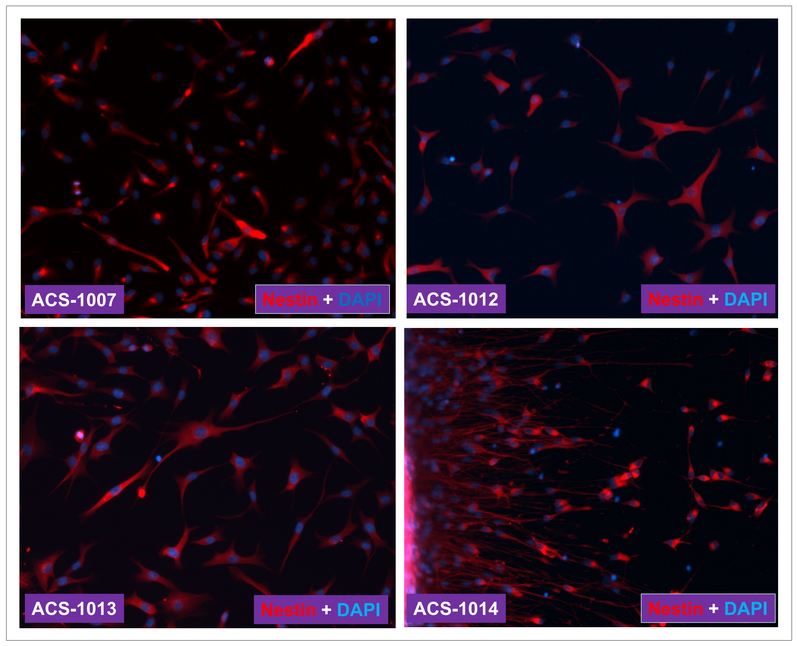


Figure 2. hiPSCs carry LRRK2 somatic mutations (R50H, I723V, M2397T) identified by exome sequencing and confirmed by direct DNA sequencing

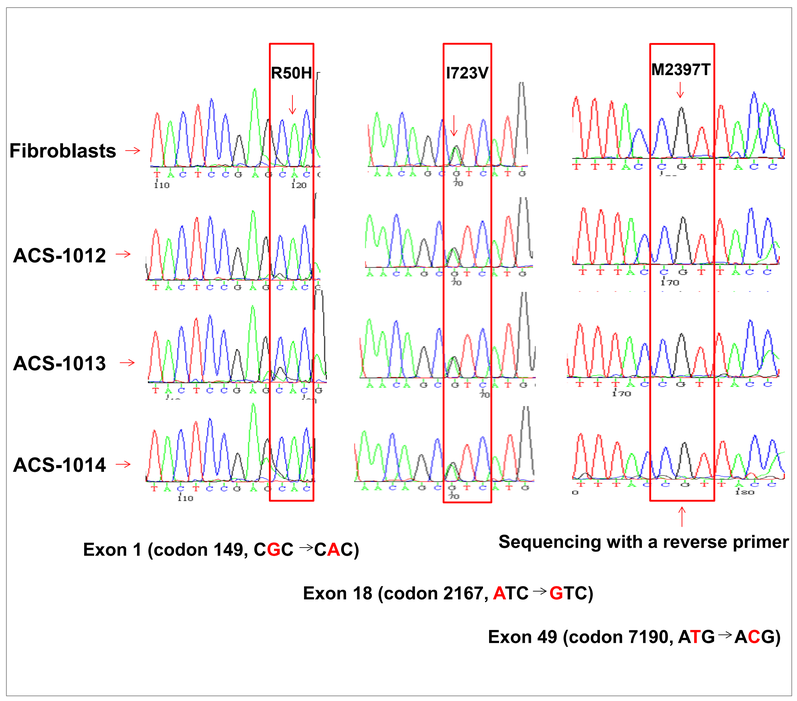


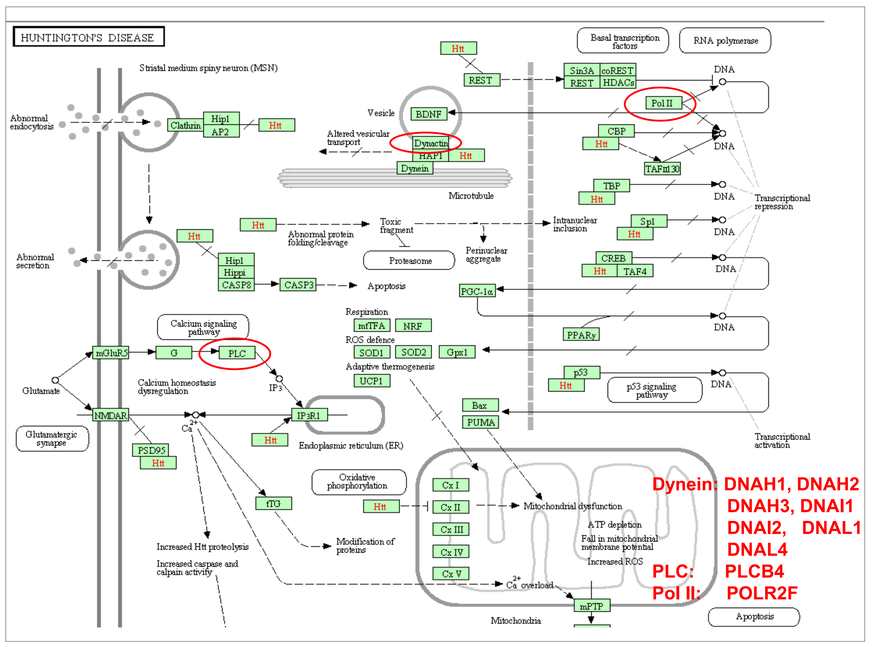
Table 1. Identification of missense mutations in Parkinson's hiPSC lines by exome sequencing

Cell line	Reprograming method	Passage #	Mutation #	Shared mutations with fibroblast	Shared mutations with ACS-1012	Shared mutations with ACS-1013	Shared mutations with ACS-1014
Parental Fibroblasts	N/A	P5	319	N/A	77%	78%	80%
ACS-1012	Retroviral	P13	325	75%	N/A	74%	77%
ACS-1013	Sendai viral	P12	310	81%	77%	N/A	83%
ACS-1014	Episomal	P16	362	71%	69%	71%	N/A

Table 2. Identification of disease-related mutations in Parkinson's hiPSC lines

Gene symbol	Protein function	Gene mutation-associated disease
NPC1	Maintaining the structural and functional integrity of nerve terminals	Autosomal recessive neurodegenerative disorder
NGF	Neural plasticity and apoptosis of neurons	Hereditary sensory and autonomic neuropathy
LRP2	HDL endocytosis	Donai-Barrow syndrome
MEF2A	Neural differentiation and survival	Autosomal dominant coronary artery disease 1
LRP2	Regulation of HDL endocytosis	Doonai-Barrow Syndrome
MEF2A	Neural differentiation and survival	Autosomal dominant coronary artery disease 1
DNAI1	Regulation of dynein activity	Primary ciliary dyskinesia and kartagener syndrome
SLC5A1	Sodium/glucose cotransporter	Glucose-galactose malabsorption
RP1L1	Differentiation of photoreceptor cells	Macular dystrophy
PNPLA2	Hydrolysis of triglycerides	Neutral lipid storage disease with myopathy
DBT	Amino acid metabolism	Maple syrup urine disease
BSND	Chloride reabsorption	Bartter syndrome
ZNF469	Regulator of collagen fibers	Cornea syndrome

Figure 3. Mutations in Dynein, PLC, and Pol II genes associated with Huntington's disease pathway



Summary

We have created three fully characterized iPSC lines that carry mutations in LRRK2 and other disease-associated genes, which enable us to better investigate the underlying mechanisms of Parkinson's and other diseases in a disease-relevant cell type, as well as to understand the potential differences observed by the reprogramming methods. We identified 228 conserved gene mutations in all 3 iPSC lines along with the parental fibroblasts and pre-existing mutations in the parental fibroblasts accounted for approximately 70% of the missense mutations in 3 iPSC lines regardless of the reprogramming method. In addition, 3 Parkinson's iPSC lines created with three different reprogramming methods have showed a similar efficiency of neural differentiation into neural progenitors and had a similar mutation rate in their exons.

References

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