

## Alpha-Synuclein Expression in the Oligodendrocyte Lineage: an In Vitro and In Vivo Study Using Rodent and Human Models

Mehdi Djelloul,<sup>1,2,3</sup> Staffan Holmqvist,<sup>1,2,3</sup> Antonio Boza-Serrano,<sup>2,4</sup> Carla Azevedo,<sup>1,2,3</sup> Maggie S. Yeung,<sup>5</sup> Stefano Goldwurm,<sup>6</sup> Jonas Frisén,<sup>5</sup> Tomas Deierborg,<sup>2,4</sup> and Laurent Roybon<sup>1,2,3,\*</sup>

<sup>1</sup>Stem Cell Laboratory for CNS Disease Modeling, Wallenberg Neuroscience Center, Department of Experimental Medical Science, BMC A10, Lund University, 22184 Lund, Sweden

<sup>2</sup>Strategic Research Area MultiPark, Lund University, 22184 Lund, Sweden

<sup>3</sup>Lund Stem Cell Center, Lund University, 22184 Lund, Sweden

<sup>4</sup>Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, BMC B11, Lund University, 22184 Lund, Sweden

<sup>5</sup>Department of Cell and Molecular Biology, Karolinska Institutet, 171 77 Stockholm, Sweden

<sup>6</sup>Parkinson Institute, Istituti Clinici di Perfezionamento, 20126 Milan, Italy

\*Correspondence: [laurent.roybon@med.lu.se](mailto:laurent.roybon@med.lu.se)

<http://dx.doi.org/10.1016/j.stemcr.2015.07.002>

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### SUMMARY

In this study, we sought evidence for alpha-synuclein (ASYN) expression in oligodendrocytes, as a possible endogenous source of ASYN to explain its presence in glial inclusions found in multiple system atrophy (MSA) and Parkinson's disease (PD). We identified ASYN in oligodendrocyte lineage progenitors isolated from the rodent brain, in oligodendrocytes generated from embryonic stem cells, and in induced pluripotent stem cells produced from fibroblasts of a healthy individual and patients diagnosed with MSA or PD, in cultures in vitro. Notably, we observed a significant decrease in ASYN during oligodendrocyte maturation. Additionally, we show the presence of transcripts in PDGFRA/CD140a<sup>+</sup> cells and SOX10<sup>+</sup> oligodendrocyte lineage nuclei isolated by FACS from rodent and human healthy and diseased brains, respectively. Our work identifies ASYN in oligodendrocyte lineage cells, and it offers additional in vitro cellular models that should provide significant insights of the functional implication of ASYN during oligodendrocyte development and disease.

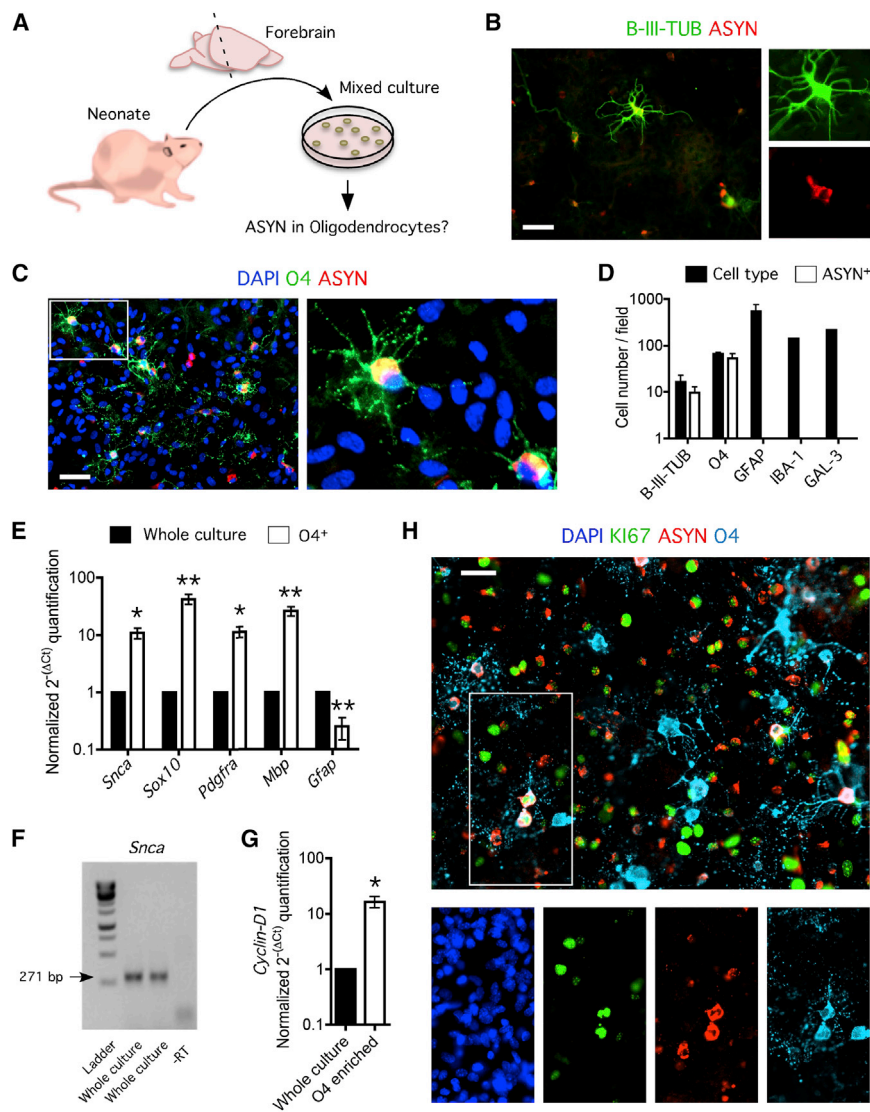
### INTRODUCTION

Multiple system atrophy (MSA) and Parkinson's disease (PD) are adult-onset progressive neurodegenerative diseases that are hallmarked at the cellular level by the presence of alpha-synuclein (ASYN) protein containing inclusions. Interestingly, the inclusions are found in neurons in PD, where the *SNCA* gene encoding for ASYN protein is expressed, whereas they are prominent in oligodendrocytes as glial cytoplasmic inclusions (GCIs) in MSA (Papp et al., 1989; Stefanova et al., 2009). Because strong evidence showing that oligodendrocytes in the adult brain are capable of expressing *SNCA* is lacking, recent studies inspired from parkinsonian experimental models have proposed the interesting hypothesis that ASYN present in GCIs in MSA could be of neuronal origin and transfer to oligodendrocytes (Kisos et al., 2012; Reyes et al., 2014), where it would accumulate and potentially lead to oligodendrocyte dysfunction. However, more than a decade ago, Richter-Landsberg and coworkers reported on the transient expression of *Snca* in cultures enriched in oligodendrocytes, prepared by mechanical shaking of mixed rat glial primary brain cultures (Richter-Landsberg et al., 2000). This finding, however, was neither confirmed nor further explored, and in vivo evidences of ASYN expression during oligodendrocyte maturation are still missing. Consequently, follow-up

studies mainly focused on understanding the functional consequences of wild-type or mutant human ASYN targeted expression in oligodendrocytes in experimental models in vivo and in vitro (Kragh et al., 2013; Yazawa et al., 2005).

To date, the origin of ASYN in GCIs in oligodendrocytes in MSA is still elusive, as is that of the few inclusions found in glial cells in the substantia nigra of people with PD (Wakabayashi et al., 2000). For these reasons, ASYN expression in oligodendrocytes remains to be firmly established, especially since investigations using patient material are still controversial (Asi et al., 2014; Miller et al., 2005). One way to explore ASYN expression in human oligodendrocytes is through the generation of human cellular models such as induced pluripotent stem cells (iPSCs), since patient oligodendrocytes are not always accessible postmortem.

Here using several murine and human cellular models, we present compelling evidence for ASYN expression during oligodendrocyte lineage cell development. Moreover, we identified both ASYN protein and transcripts in purified PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors and oligodendrocyte lineage SOX10<sup>+</sup> nuclei, isolated by fluorescence-activated cell sorting (FACS) from the rodent wild-type brain and human healthy and diseased brains, respectively, therefore substantiating a functional role for ASYN in oligodendrocytes.



**Figure 1. Localization of ASYN in Mouse Primary Neurons and Pre-oligodendrocyte Progenitors**

(A) Experimental approach used for generating rodent postnatal primary forebrain cultures is shown.

(B) Rodent postnatal primary forebrain cultures aged 25 DIV contain B-III-TUB<sup>+</sup> neurons expressing *Snca*. The scale bar represents 50  $\mu$ m.

(C) Rodent postnatal primary forebrain cultures contain O4<sup>+</sup> oligodendrocytes expressing ASYN. Nuclei are counterstained with DAPI. (Right) Higher magnification of the region of interest is shown. The scale bar represents 50  $\mu$ m.

(D) Quantification of neurons (B-III-TUB<sup>+</sup>), oligodendrocytes (O4<sup>+</sup>), astrocytes (GFAP<sup>+</sup>), and microglia (IBA1<sup>+</sup> and GAL-3<sup>+</sup>) co-expressing ASYN (white bars). Black bars represent quantification for each cell type. Values are indicated as the number of cells per field of view. Mean  $\pm$  SEM; n = 3 independent cultures.

(E) Quantitative real-time PCR analysis shows the abundance (>10-fold) of *Snca* transcripts in FACS-purified O4<sup>+</sup> oligodendrocyte samples compared to whole primary culture sample. Oligodendrocyte enrichment is assessed by the quantification of oligodendrocyte-specific marker transcripts and the absence of astrocytic *Gfap* transcripts (see [Supplemental Experimental Procedures](#)). Mean  $\pm$  SEM; n = 3–4 independent experiments; t test, \*p < 0.05, \*\*p < 0.01.

(F) The set of primers we designed to assess mouse *Snca* expression by quantitative real-time PCR recognizes a unique product of 271 bp and was tested on two independent cultures.

(G) Quantitative real-time PCR analysis shows significant enrichment in *Cyclin-D1* transcripts in purified O4<sup>+</sup> oligodendrocyte sample compared to whole primary culture sample. Mean  $\pm$  SEM; n = 3 independent experiments; t test, \*p < 0.05.

(H) Identification of KI67<sup>+</sup>/O4<sup>+</sup>/ASYN<sup>+</sup> oligodendrocyte in rodent postnatal primary forebrain culture. Nuclei are counterstained with DAPI. Insets show higher magnification of the region indicated with straight lines. The scale bar represents 50  $\mu$ m.

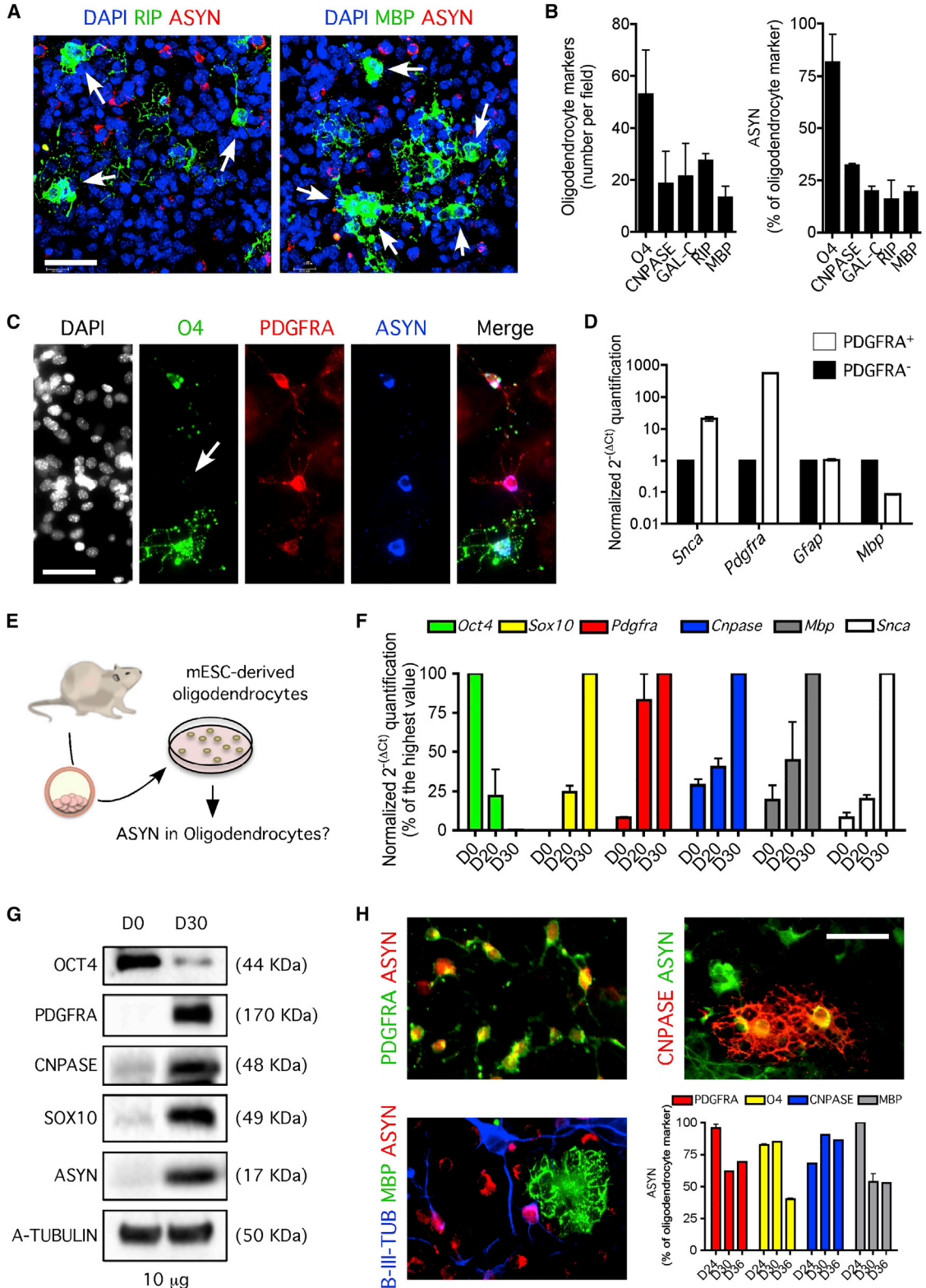
## RESULTS

### ASYN Protein and Transcripts Identified in O4<sup>+</sup> Oligodendrocytes in Mouse Brain Primary Cultures

Oligodendrocytes are abundantly produced during development and at neonatal age (Kessaris et al., 2006). The postnatal forebrain, therefore, should provide a rapid source of oligodendrocytes at different stages of maturation for experimental investigations in vitro.

We performed immunocytochemistry (ICC) on dissociated cell cultures from mouse neonates, grown for

3 weeks in vitro (Figure 1A). These cultures were composed of beta-III-tubulin (B-III-TUB)-expressing neurons ( $\approx$ 2%), glial-fibrillary acidic protein (GFAP)-expressing astrocytes ( $\approx$ 75%), IBA1-expressing microglia ( $\approx$ 15%), and oligodendrocyte lineage-specific glycolipid antigen (O4)-positive oligodendrocytes ( $\approx$ 8%) (Figures 1B–1D; Figure S1A). Importantly, ICC for each of these cell-type-specific markers and ASYN showed specific localization of ASYN in B-III-TUB<sup>+</sup> neurons and in O4<sup>+</sup> oligodendrocytes (Figures 1B–1D; Figures S1A and S1B).



(legend on next page)



We isolated O4<sup>+</sup> oligodendrocytes by FACS from the rest of the cells present in our primary cultures, and we measured the abundance of total *Snca* transcripts by quantitative real-time PCR. Consistent over multiple rounds of experiments, our quantitative real-time PCR data clearly showed the presence of *Snca* transcripts (>10-fold increase relative to the whole culture) in the O4<sup>+</sup>-purified samples (Figures 1E and 1F), therefore suggesting that O4<sup>+</sup> oligodendrocytes indeed do express the *Snca* gene encoding for ASYN.

Interestingly, we identified ASYN protein and *Snca* transcripts in cycling oligodendrocytes, probably oligodendrocyte progenitors (Figures 1G and 1H).

### Decreased ASYN Expression during Oligodendrocyte Maturation

To become mature and capable of myelinating axons, oligodendrocytes undergo different stages of maturation, each characterized by temporal expression of specific markers (Zhang, 2001).

Interestingly, while we previously found that >90% of O4<sup>+</sup> immature oligodendrocytes present in mice primary postnatal forebrain cultures co-expressed ASYN, we observed a strong reduction (up to 70% less compared to O4<sup>+</sup> oligodendrocytes) in co-expression of ASYN and the mature oligodendrocyte markers 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPASE, <40%), galactocerebroside (GAL-C, <35%), and myelin basic protein (MBP, <25%), clearly identifiable by confocal microscopy (Figures 2A and 2B; Figure S2). In marked contrast, ASYN expression was high in immature platelet-derived growth factor receptor alpha-positive (PDGFRA) oligodendrocyte progenitors, measured

by ICC and quantitative real-time PCR (Figures 2C and 2D). Our data, therefore, suggest that ASYN expression decreases during oligodendrocyte maturation and is present as early as PDGFRA<sup>+</sup> oligodendrocyte progenitor stage.

To ascertain that ASYN expression was specific to oligodendrocyte development and not the result of a transient expression due to the passage of cells isolated from the brain to an in vitro culture system, we investigated ASYN expression during the generation of oligodendrocytes from mouse embryonic stem cells (mESCs; Figure 2E). We derived ESC from wild-type mice, which we differentiated into oligodendrocytes using a previously published protocol (Czepiel et al., 2011). We first ascertained the downregulation and upregulation of pluripotent marker Oct4 and oligodendrocyte lineage-specific markers SRY (sex-determining region Y)-box 10 (SOX10), PDGFRA, CNPASE, and MBP, respectively, by quantitative real-time PCR, western blot, and ICC (Figures 2F–2H; data not shown), during mESC differentiation. We next confirmed the abundance of *Snca* transcripts and ASYN protein in our cultures, by quantitative real-time PCR, western blot, and ICC (Figures 2F–2H). Importantly, we found that the majority of O4<sup>+</sup> oligodendrocytes expressed *Snca* (Figure 2H), and that, yet again, ASYN decreases during oligodendrocyte maturation (Figure 2H).

### Oligodendrocytes Generated from iPSCs from a Healthy Individual and Patients with PD or MSA Also Express SNCA

Because ASYN expression in oligodendrocytes from MSA patients is debated and that in oligodendrocytes of people

#### Figure 2. ASYN Expression Decreases during Oligodendrocyte Maturation

(A) Absence of ASYN in RIP<sup>+</sup> and MBP<sup>+</sup> oligodendrocytes detected by confocal microscopy. White arrows pinpoint oligodendrocyte cell bodies. The scale bar represents 50  $\mu$ m.

(B) Rodent postnatal primary forebrain cultures contain O4<sup>+</sup> immature oligodendrocytes ( $\approx$  70% of total oligodendrocyte markers) and CNPASE<sup>+</sup>, GAL-C<sup>+</sup>, and MBP<sup>+</sup> (<20% for all three markers) mature oligodendrocytes. Mean  $\pm$  SEM; n = 2–3 independent experiments. There is decreased co-expression between ASYN and CNPASE, GAL-C, or MBP compared to O4<sup>+</sup> oligodendrocytes. Mean  $\pm$  SEM; n = 2–3 independent experiments.

(C) Rodent postnatal primary forebrain cultures contain PDGFRA<sup>+</sup> oligodendrocyte progenitors expressing ASYN. Nuclei are counterstained with DAPI. White arrowhead pinpoints an O4<sup>-</sup>/ASYN<sup>+</sup>/PDGFRA<sup>+</sup> oligodendrocyte progenitor. The scale bar represents 50  $\mu$ m.

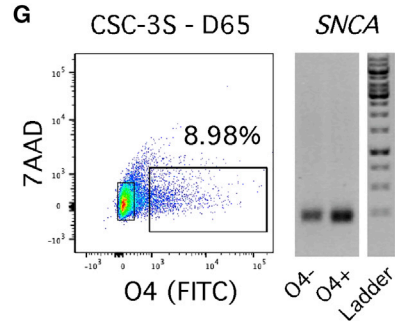
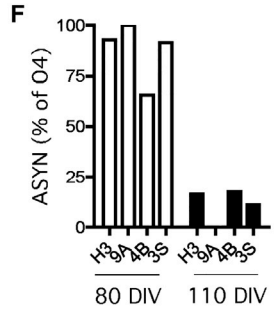
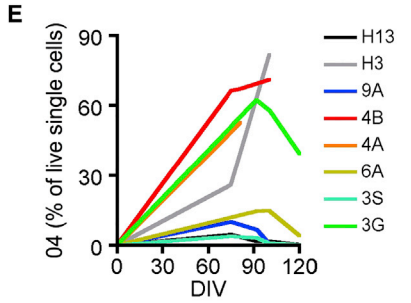
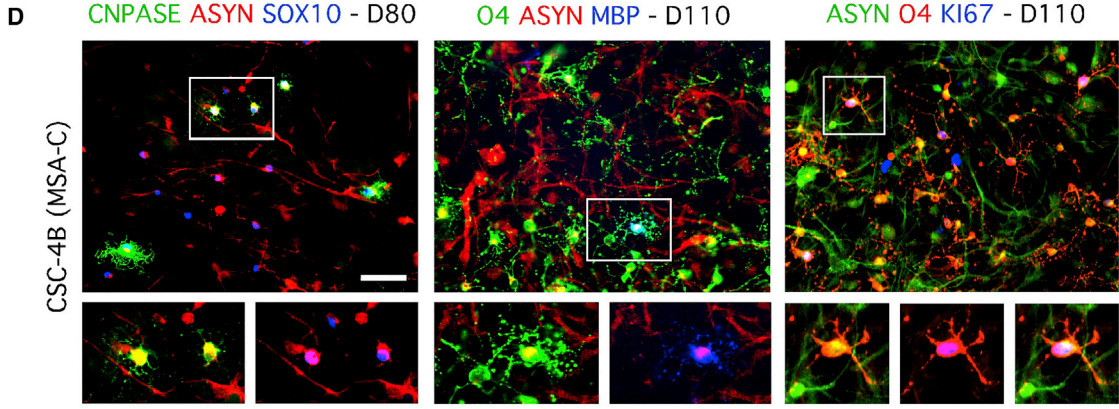
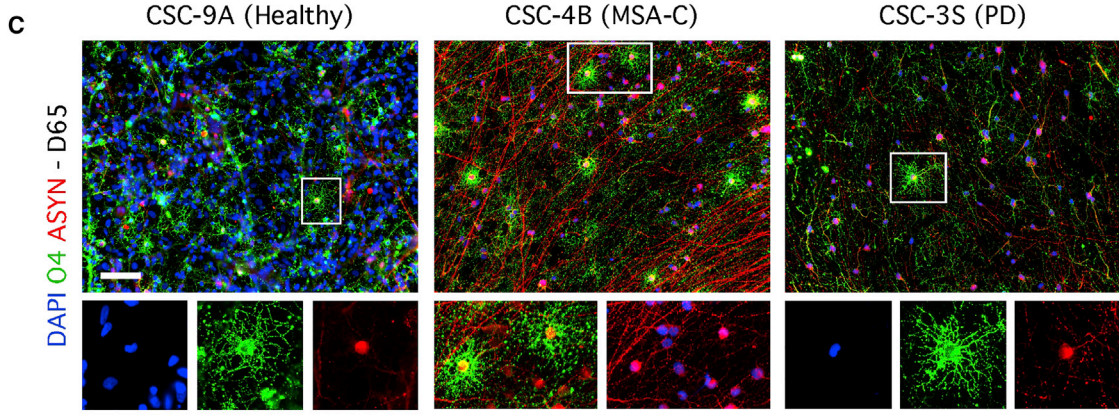
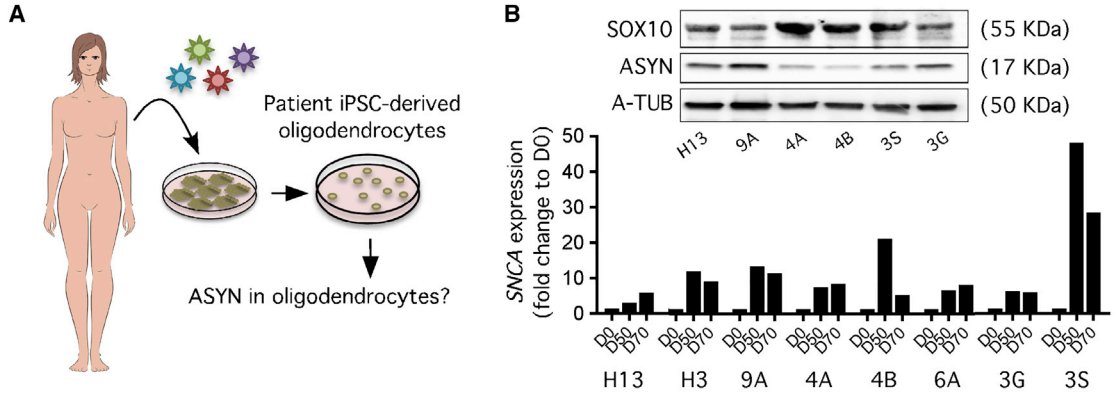
(D) Quantitative real-time PCR analysis shows the abundance (>10-fold) of *Snca* transcripts in FACS-purified PDGFRA<sup>+</sup> oligodendrocyte progenitor samples compared to PDGFRA<sup>+</sup> oligodendrocyte progenitor-depleted samples. Enrichment in oligodendrocyte progenitors is assessed by the quantification of *Pdgfra* transcripts and the absence of astrocytic *Gfap* and mature oligodendrocytic *Mbp* transcripts. Mean  $\pm$  SEM; n = 2–3 independent experiments.

(E) Experimental approach used for assessing ASYN expression during rodent oligodendrocyte development. The mESCs were derived from a blastocyst aged E3.5 and subsequently differentiated into oligodendrocytes for a period of 4–5 weeks in vitro.

(F) Quantitative real-time PCR shows the loss of *Oct4* and upregulation of *Nkx2.2*, *Pdgfra*, *Cnpase* and *Mbp*, and *Snca* during the differentiation of mESCs into oligodendrocytes. For each marker, values are presented as a percentage of the highest value. Mean  $\pm$  SEM; n = 2 independent experiments.

(G) Western blotting confirms the downregulation of OCT4 and expression of the oligodendroglial markers and ASYN during the differentiation of mESCs into oligodendrocytes. Blot is representative of two independent experiments.

(H) ICC on mESC-differentiated cultures aged 36 DIV confirms the presence and absence of ASYN in PDGFRA<sup>+</sup> and CNPASE<sup>+</sup>, and MBP<sup>+</sup> oligodendrocytes, respectively. Quantifications are based on two differentiations. Images are representative of four independent differentiations. Scale bars represent 50  $\mu$ m.



(legend on next page)



with PD is still elusive, we generated iPSCs from fibroblasts (Figure 3A) of MSA patients diagnosed with cerebellar ataxia (MSA-C, patient 4) or parkinsonism (MSA-P, patient 6), a patient diagnosed with a familial form of PD (patient 3), and a healthy individual (patient 9), and assessed ASYN expression in oligodendrocyte lineage cells (Figure 3A). All iPSC clones selected were expanded and cryopreserved. Random clones were selected and processed for further analysis. Thus, clones CSC-3A, B, G, and S; CSC-4A and B; CSC-6A; and CSC-9A and B underwent rigorous characterization through a battery of tests (summarized in Figure S3), which indicated that we had effectively generated iPSCs.

We attempted to generate oligodendrocytes from our iPSC lines. To rule out the possibility of chromosomal abnormalities influencing ASYN expression in iPSC-derived oligodendrocytes, we selected one clone per patient-specific iPSC line presenting a normal karyotype to carry out most of our investigations. We devised a protocol, modified from that of Stacpoole and coworkers (Stacpoole et al., 2013), where we employed dual inhibition of SMAD signaling by small molecules LDN and SB to neutralize our cultures, sonic hedgehog-mediated ventral patterning, and PDGF-AA/IGF1/NT3/T3/HGF-mediated terminal differentiation. This modified protocol, proven efficient when applied to the human ESC lines H13 and H3 (Figure S4; data not shown), successfully produced oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes from iPSCs. Thus, by 60 days in vitro (DIV), nearly all SOX10<sup>+</sup> cells co-expressed NKX2.2 and OLIG2, NG2, and PDGFRA and O4 (Figures S5A–S5C and S6A), and those cells exhibited in majority an immature-bipolar morphology where ASYN could clearly be identified by confocal microscopy, quantitative real-time PCR, and western blot (Figures 3B and 3C; Figures S5 and S6). Additionally, ASYN could be detected in neurons (Figure S5D), whereas it was absent in astrocytes (Fig-

ure S5E). Interestingly, from 65 DIV onward, O4<sup>+</sup> oligodendrocytes displayed a perinuclear localization of ASYN (Figures 3D and 3E), as we observed for mouse O4<sup>+</sup> oligodendrocytes (Figures 1 and 2). In cultures aged 110 DIV, ASYN expression could still be detected in a few CNPASE<sup>+</sup> and MBP<sup>+</sup> mature oligodendrocytes (Figure 3D), sometimes as a perinuclear dot (Figure S6C; data not shown). Interestingly, the disease lines CSC-4A, CSC-4B, and CSC-3G gave rise to an accelerated high-yield generation of O4<sup>+</sup> oligodendrocytes (up to 70% of live single cells) in 70 DIV (Figure 3E). Finally, we confirmed the decrease in ASYN in O4<sup>+</sup> oligodendrocytes over time (Figure 3F) and the presence of SNCA transcripts in FACS-purified O4<sup>+</sup> oligodendrocytes from the line CSC-3S aged 80 DIV (Figure 3G).

### ASYN Protein and Transcripts Identified in Oligodendrocytes from Mouse and Human Brains

We identified *Snca* transcripts in PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors freshly isolated by FACS from embryonic day (E)18 mouse brains (Figures 4A–4C). Importantly, we detected ASYN by ICC in nearly all oligodendrocytes present in homogenous cultures resulting from the isolation of E17.5 and E18 PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors (Figure 4D), ruling out the unlikely possibility that ASYN may have been transferred from neurons to oligodendrocytes in primary cultures (Figure 1). When these progenitors matured into O4<sup>+</sup> oligodendrocytes (Sim et al., 2011), ASYN was still present, with the exception of a few oligodendrocytes having a more complex morphology (Figure 4D).

Finally, we took advantage of a previously developed technique (Ernst et al., 2014; Yeung et al., 2014), to isolate human oligodendrocyte lineage nuclei by FACS, to assess the presence of SNCA transcripts (Figures 4E–4I). We prepared nuclei samples from the pons of snap-frozen postmortem tissue from the following six patients: three

### Figure 3. ASYN in iPSC-Derived O4<sup>+</sup> Oligodendrocytes

(A) Experimental approach used for generating and assessing ASYN expression during human iPSC-derived oligodendrocyte development. Healthy individual, MSA-C, MSA-P, and PD patient's skin fibroblasts were reprogrammed by means of viral overexpression of stemness factors hKLF4, hC-MYC, hOCT4, and hSOX2. Following characterization, human iPSCs were subsequently differentiated into oligodendrocytes over a period of 110 DIV.

(B) Western blotting confirms the expression of the oligodendroglial marker SOX10 and ASYN during the differentiation of human iPSCs into oligodendrocytes. Blot is representative of two independent experiments. Quantitative real-time PCR shows the upregulation of SNCA in iPSCs during oligodendrocyte differentiation.

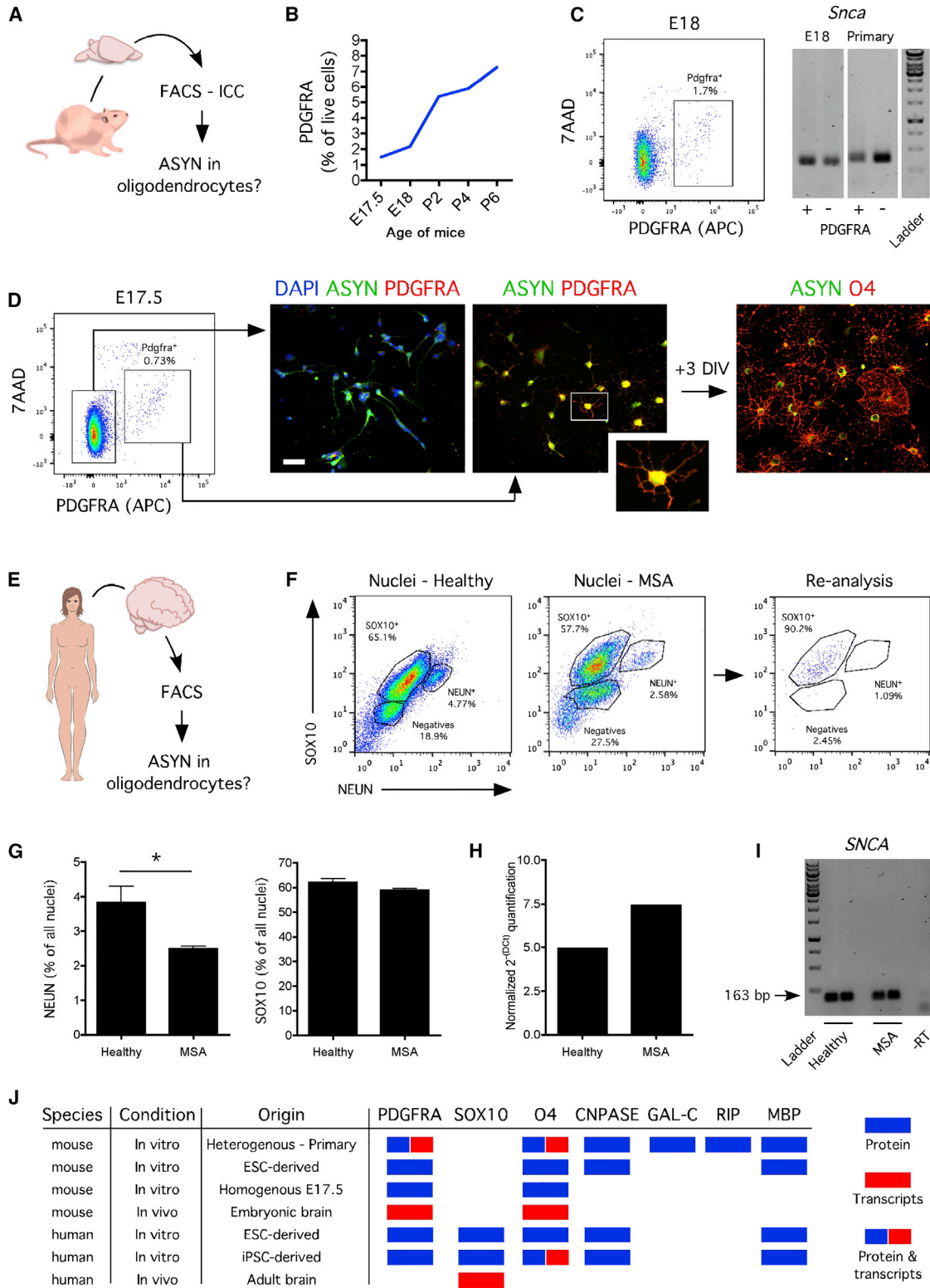
(C) Perinuclear localization of ASYN in immature O4<sup>+</sup> oligodendrocytes aged 65 DIV revealed by ICC. Images are representative of two to three independent differentiations. Scale bars represent 50  $\mu$ m.

(D) Presence of ASYN in O4<sup>+</sup>, CNPASE<sup>+</sup>, and MBP<sup>+</sup> oligodendrocytes aged 110 DIV revealed by ICC. (Middle, bottom left inset) O4<sup>+</sup>/MBP<sup>-</sup>/ASYN<sup>-</sup> and O4<sup>+</sup>/MBP<sup>+</sup>/ASYN<sup>+</sup> oligodendrocytes are shown. Scale bars represent 50  $\mu$ m.

(E) Quantification of O4<sup>+</sup> oligodendrocytes by flow cytometry for eight PSC lines differentiated side by side. The early emergence of O4<sup>+</sup> oligodendrocytes from line 4 was observed in separate rounds of differentiation.

(F) Quantification of O4<sup>+</sup> oligodendrocytes co-expressing ASYN over time is shown.

(G) Quantitative real-time PCR shows SNCA transcripts in 3S iPSC-derived O4<sup>+</sup> oligodendrocyte samples, purified by FACS.



(legend on next page)



healthy and three diagnosed with MSA with confirmed GCIs in oligodendrocytes (Figure S7A). We isolated from each sample the oligodendrocyte lineage SOX10<sup>+</sup> nuclei and quantified neuronal NEUN<sup>+</sup> nuclei by FACS (Figure 4G; Figure S7B). We detected a lower proportion of neurons in the pons region of the MSA patients (Figure 4H), confirming the MSA diagnosis; we also found a lower percentage of SOX10<sup>+</sup> nuclei in the diseased samples, although not significant (Figure 4H). Importantly, we could detect the presence of *SNCA* transcripts in oligodendrocyte lineage nuclei of two samples isolated from the brain of a healthy and an MSA patient (Figures 4H and 4I), as described by Asi and coworkers (Asi et al., 2014).

## DISCUSSION

The absence of *SNCA* transcripts in oligodendrocytes in the adult brain of healthy and MSA patients has been reported repeatedly (Iwai et al., 1995; Miller et al., 2005; Solano et al., 2000), but recent studies have challenged these results. Because MSA has been identified as a non-genetic disorder, it is difficult to propose an explanation for the origin of ASYN in GCIs present in oligodendrocytes under pathological condition. This has led to the emergence of a hypothesis advocating a mechanism of cell-to-cell transfer of exogenous ASYN to oligodendrocytes, similar to that experimentally established from neuron-to-neuron in PD models (Hansen et al., 2011). However, the recent use of more advanced techniques, such as laser-capture micro-dissection, has allowed for the isolation of adult white matter glia from human post-mortem tissue, resulting in the detection of *SNCA* tran-

scripts from MSA, idiopathic PD, and control cases (Asi et al., 2014).

We clearly identified ASYN protein by ICC and western blotting and we detected transcripts by quantitative real-time PCR in oligodendrocyte lineage cells from rodent and human origins (summarized in Figure 4J). We could not detect ASYN expression in astrocytes and microglia, which are cell types known to take up exogenous ASYN (Boza-Serrano et al., 2014; Lee et al., 2010). Moreover, the few neurons present in cultures combined with the multitude of media changes applied during the culture period, and the discovery of *Snca*/*SNCA* transcripts in freshly isolated oligodendrocytes and ASYN protein in homogenous cultures of freshly isolated, maturing PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors, together allow us to rule out the possibility that healthy or dying neurons, even contaminating neurons, may have released large quantities of ASYN, which would accumulate, not being sufficiently degraded, in the oligodendrocytes only. Moreover, we showed relocation of ASYN during oligodendrocyte differentiation from the processes to the perinuclear space, where the protein appears as a single dot-like structure suggesting it is finally degraded.

We identified *SNCA* transcripts in human SOX10<sup>+</sup> oligodendrocyte lineage nuclei samples isolated from healthy and MSA patients (Figure 4). This result is in line with recent observations (Asi et al., 2014). Whether ASYN expression identified in these two studies is reminiscent of a basal expression in quiescent OPCs, and the difference although not significant identified in oligodendrocytes from MSA and control samples is the result of a greater oligodendrogenesis probably triggered by neuronal injury (Ahmed et al., 2013) remain to be determined.

We found a decrease of ASYN associated with oligodendrocyte maturation characterized by complex cellular

### Figure 4. ASYN in Oligodendrocytes In Vivo

- (A) Schematic representation shows the experimental approach used for assessing ASYN in the mouse embryonic brain.
- (B) Quantification of PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors and O4<sup>+</sup> oligodendrocytes from the rodent brain at embryonic and postnatal stages, measured by FACS, is shown.
- (C) Loading of quantitative real-time PCR product confirms the presence of *Snca* transcripts in E18 PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors, freshly isolated by FACS, from the mouse embryonic brain.
- (D) Detection of ASYN in homogenous cultures from mouse embryonic PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors isolated by FACS at E17.5. ASYN is maintained in homogenous cultures of PDGFRA/CD140a<sup>+</sup> oligodendrocyte progenitors maturing into O4<sup>+</sup> oligodendrocytes. The scale bar represents 50  $\mu$ m.
- (E) Schematic representation shows the experimental approach used for assessing ASYN expression in oligodendrocyte lineage nuclei isolated from the brain of healthy and MSA patients.
- (F) FACS plots represent the gating strategy employed for isolating SOX10<sup>+</sup> oligodendrocyte lineage nuclei from the samples.
- (G) The decrease in the percentage of NEUN<sup>+</sup> nuclei of the total population of all nuclei, in the pons region of MSA patients (n = 3) compared to control (n = 3), was measured by FACS. Mean  $\pm$  SEM; t test, \*p < 0.05.
- (H) Quantitative real-time PCR shows *SNCA* transcripts in SOX10<sup>+</sup> oligodendrocyte lineage nuclei samples purified by FACS from patients T172 and T4640.
- (I) Loading of quantitative real-time PCR product confirms the presence of *SNCA* transcripts. The set of primers used to assess human *SNCA* expression by quantitative real-time PCR recognizes a unique product of 163 bp (from Asi et al., 2014).
- (J) A summary of our findings is shown.





processes and expression of the mature marker MBP. Interestingly, overexpression of ASYN in OPCs delays their maturation into MBP<sup>+</sup> oligodendrocytes (Ettle et al., 2014). Additionally, we found that ASYN is decreased in pre-myelinating oligodendrocytes, regardless of the genetic background of the iPSC (diseased or healthy). This observation, although made in vitro, may allow us to rule out that the origin of ASYN in GCIs in MSA, and glial inclusions in PD, may result from the accumulation of a lifetime production of ASYN throughout glial lineages.

Successful generation of patient-specific iPSCs, and their subsequent differentiation into brain cell types, has opened up unlimited access of patient brain cells to a growing number of investigators interested in studying neurodegenerative diseases. Here we have pioneered the generation of oligodendrocytes from MSA and familial PD iPSC lines. Our protocol for generating oligodendrocytes from human iPSCs, therefore, adds on to those recently developed for studying demyelination diseases (Douvaras et al., 2014; Numasawa-Kuroiwa et al., 2014; Piao et al., 2015; Stacpoole et al., 2013; Wang et al., 2013). Importantly, our iPSC models provide a greater number of lines available for studying synucleinopathies.

## EXPERIMENTAL PROCEDURES

### Use of Animals and Human Samples

All procedures were conducted in accordance with national and European Union directives. The derivation of mESC and generation of human iPSC lines using viral-mediated gene delivery were approved by the ethical committee for the use of laboratory animals at Lund University and the Swedish Work Environment Authority.

### Primary Cell Cultures

Primary forebrain cultures were prepared from 1- to 3-day-old wild-type mice and cultured in DMEM (Life Technologies) supplemented with fetal bovine serum (FBS, 10% v/v) and P/S (1% v/v). After 3 weeks, cells were detached using trypsin, either for FACS analysis or to be seeded on 96-well plates.

### Oligodendrocyte Differentiation from Mouse and Human PSCs

The mESC-derived oligodendrocytes were generated using a published protocol (Czepiel et al., 2011). Human PSC (hPSC)-derived oligodendrocytes were generated using a protocol modified from a previously published study (Stacpoole et al., 2013).

### FACS

A FACSAria-III was used for cell sorting and analysis (MultiPark core facility).

### Immunostainings and Image Acquisition

ICC was carried out using standard protocols. Images were acquired using an inverted epifluorescent microscope LRI-Olympus IX-73 and a confocal microscope Leica SP8 (MultiPark core facility).

### Statistical Analyses

All quantitative data were analyzed using Prism 6.0 (GraphPad). Sample groups were subjected to un-paired t test.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.stemcr.2015.07.002>.

## AUTHOR CONTRIBUTIONS

M.D. performed experiments and analysis. S.H. assisted with reprogramming and characterization of iPSC lines. A.B.-S. assisted with primary cultures and western blotting. C.A. assisted with the differentiation of mESCs and hPSCs and stainings. M.S.Y. assisted with nuclei purification. L.R. assisted with the derivation of mESCs, characterization of iPSC lines, and analysis. T.D., J.F., and S.G. provided reagents and input on experiments. M.D. and L.R. conceived the experiments and wrote the manuscript. All authors gave input on the manuscript and approved its final version.

## ACKNOWLEDGMENTS

We are deeply thankful to V. Fossati and N. Lamas for helpful critical reading of the manuscript and to J. Goldman for generously providing the O4 antibody. We thank J.-Y. Li for providing access to wild-type mice for deriving mESCs; A. Collin for helping with karyotyping interpretation; C. Vignon for helping with the maintenance of the mESCs; A. Hammarberg, S. Giatrellis, and M. Toro for FACS advice; and J. Bigarreau for helping with graphic illustrations. We are also thankful to P.H. Jensen for constructive feedback during the editing of the manuscript. Human fibroblast cell lines were obtained from Coriell, the Cell Line and DNA Biobank from Patients affected by Genetic Diseases (Istituto G. Gaslini), and the Parkinson Institute Biobank (<http://www.parkinsonbiobank.com>), members of the Telethon Network of Genetic Biobanks (project GTB12001) funded by Telethon Italy. We are thankful to the Brain Bank at Columbia University team for providing us with the human postmortem tissue samples. This work was supported by the Strong Research Environment MultiPark (multidisciplinary research on Parkinson's disease at Lund University) and through external grants to L.R. from the Jeansson's foundation; the Swedish Parkinson foundation (Parkinsonfonden); the Holger Crafoord foundation; the Segerfalk foundation; the Åke Wibergs foundation; the Greta och Johan Kocks foundation; and donations for science, medicine, and technology at Fysiografen in Lund. L.R. is a young investigator supported by MultiPark-Strategic Research Environment at Lund University, funded by the Swedish government, and partner of BrainStem-Stem Cell Center of Excellence in Neurology, funded by Innovation Fund Denmark.



Received: December 7, 2014

Revised: July 6, 2015

Accepted: July 6, 2015

Published: July 30, 2015

## REFERENCES

- Ahmed, Z., Asi, Y.T., Lees, A.J., Revesz, T., and Holton, J.L. (2013). Identification and quantification of oligodendrocyte precursor cells in multiple system atrophy, progressive supranuclear palsy and Parkinson's disease. *Brain Pathol.* 23, 263–273.
- Asi, Y.T., Simpson, J.E., Heath, P.R., Wharton, S.B., Lees, A.J., Revesz, T., Houlden, H., and Holton, J.L. (2014). Alpha-synuclein mRNA expression in oligodendrocytes in MSA. *Glia* 62, 964–970.
- Boza-Serrano, A., Reyes, J.F., Rey, N.L., Leffler, H., Bousset, L., Nilsson, U., Brundin, P., Venero, J.L., Burguillos, M.A., and Deierborg, T. (2014). The role of Galectin-3 in  $\alpha$ -synuclein-induced microglial activation. *Acta Neuropathol. Commun.* 2, 156.
- Czepiel, M., Balasubramanian, V., Schaafsma, W., Stancic, M., Mikkers, H., Huisman, C., Boddeke, E., and Copray, S. (2011). Differentiation of induced pluripotent stem cells into functional oligodendrocytes. *Glia* 59, 882–892.
- Douvaras, P., Wang, J., Zimmer, M., Hanchuk, S., O'Bara, M.A., Sadiq, S., Sim, F.J., Goldman, J., and Fossati, V. (2014). Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. *Stem Cell Reports* 3, 250–259.
- Ernst, A., Alkass, K., Bernard, S., Salehpour, M., Perl, S., Tisdale, J., Possnert, G., Druid, H., and Frisén, J. (2014). Neurogenesis in the striatum of the adult human brain. *Cell* 156, 1072–1083.
- Ettle, B., Reiprich, S., Deusser, J., Schlachetzki, J.C., Xiang, W., Prots, I., Masliah, E., Winner, B., Wegner, M., and Winkler, J. (2014). Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. *Mol. Cell. Neurosci.* 62, 68–78.
- Hansen, C., Angot, E., Bergström, A.L., Steiner, J.A., Pieri, L., Paul, G., Outeiro, T.F., Melki, R., Kallunki, P., Fog, K., et al. (2011).  $\alpha$ -Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J. Clin. Invest.* 121, 715–725.
- Iwai, A., Masliah, E., Yoshimoto, M., Ge, N., Flanagan, L., de Silva, H.A., Kittel, A., and Saitoh, T. (1995). The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron* 14, 467–475.
- Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., and Richardson, W.D. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat. Neurosci.* 9, 173–179.
- Kisos, H., Pukaß, K., Ben-Hur, T., Richter-Landsberg, C., and Sharon, R. (2012). Increased neuronal  $\alpha$ -synuclein pathology associates with its accumulation in oligodendrocytes in mice modeling  $\alpha$ -synucleinopathies. *PLoS ONE* 7, e46817.
- Kragh, C.L., Fillon, G., Gysbers, A., Hansen, H.D., Neumann, M., Richter-Landsberg, C., Haass, C., Zalc, B., Lubetzki, C., Gai, W.P., et al. (2013). FAS-dependent cell death in  $\alpha$ -synuclein transgenic oligodendrocyte models of multiple system atrophy. *PLoS ONE* 8, e55243.
- Lee, H.J., Suk, J.E., Patrick, C., Bae, E.J., Cho, J.H., Rho, S., Hwang, D., Masliah, E., and Lee, S.J. (2010). Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J. Biol. Chem.* 285, 9262–9272.
- Miller, D.W., Johnson, J.M., Solano, S.M., Hollingsworth, Z.R., Standaert, D.G., and Young, A.B. (2005). Absence of alpha-synuclein mRNA expression in normal and multiple system atrophy oligodendroglia. *J. Neural Transm.* 112, 1613–1624.
- Numasawa-Kuroiwa, Y., Okada, Y., Shibata, S., Kishi, N., Akamatsu, W., Shoji, M., Nakanishi, A., Oyama, M., Osaka, H., Inoue, K., et al. (2014). Involvement of ER stress in dysmyelination of Pelizaeus-Merzbacher Disease with PLP1 missense mutations shown by iPSC-derived oligodendrocytes. *Stem Cell Reports* 2, 648–661.
- Papp, M.I., Kahn, J.E., and Lantos, P.L. (1989). Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). *J. Neurol. Sci.* 94, 79–100.
- Piao, J., Major, T., Auyeung, G., Policarpio, E., Menon, J., Droms, L., Gutin, P., Uryu, K., Tchiew, J., Soulet, D., and Tabar, V. (2015). Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation. *Cell Stem Cell* 16, 198–210.
- Reyes, J.F., Rey, N.L., Bousset, L., Melki, R., Brundin, P., and Angot, E. (2014). Alpha-synuclein transfers from neurons to oligodendrocytes. *Glia* 62, 387–398.
- Richter-Landsberg, C., Gorath, M., Trojanowski, J.Q., and Lee, V.M. (2000). alpha-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *J. Neurosci. Res.* 62, 9–14.
- Sim, F.J., McClain, C.R., Schanz, S.J., Protack, T.L., Windrem, M.S., and Goldman, S.A. (2011). CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. *Nat. Biotechnol.* 29, 934–941.
- Solano, S.M., Miller, D.W., Augood, S.J., Young, A.B., and Penney, J.B., Jr. (2000). Expression of alpha-synuclein, parkin, and ubiquitin carboxy-terminal hydrolase L1 mRNA in human brain: genes associated with familial Parkinson's disease. *Ann. Neurol.* 47, 201–210.
- Stacpoole, S.R., Spitzer, S., Bilican, B., Compston, A., Karadottir, R., Chandran, S., and Franklin, R.J. (2013). High yields of oligodendrocyte lineage cells from human embryonic stem cells at physiological oxygen tensions for evaluation of translational biology. *Stem Cell Reports* 1, 437–450.
- Stefanova, N., Bücke, P., Duerr, S., and Wenning, G.K. (2009). Multiple system atrophy: an update. *Lancet Neurol.* 8, 1172–1178.
- Wakabayashi, K., Hayashi, S., Yoshimoto, M., Kudo, H., and Takahashi, H. (2000). NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol.* 99, 14–20.
- Wang, S., Bates, J., Li, X., Schanz, S., Chandler-Militello, D., Levine, C., Maherali, N., Studer, L., Hochedlinger, K., Windrem, M., and Goldman, S.A. (2013). Human iPSC-derived oligodendrocyte



progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* 12, 252–264.

Yazawa, I., Giasson, B.I., Sasaki, R., Zhang, B., Joyce, S., Uryu, K., Trojanowski, J.Q., and Lee, V.M. (2005). Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. *Neuron* 45, 847–859.

Yeung, M.S., Zdunek, S., Bergmann, O., Bernard, S., Salehpour, M., Alkass, K., Perl, S., Tisdale, J., Possnert, G., Brundin, L., et al. (2014). Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell* 159, 766–774.

Zhang, S.C. (2001). Defining glial cells during CNS development. *Nat. Rev. Neurosci.* 2, 840–843.