# NURR1 in Parkinson disease—from pathogenesis to therapeutic potential

*Mickael Decressac, Nikolaos Volakakis, Anders Björklund and Thomas Perlmann*

**Abstract** | In Parkinson disease (PD), affected midbrain dopamine (DA) neurons lose specific dopaminergic properties before the neurons die. How the phenotype of DA neurons is normally established and the ways in which pathology affects the maintenance of cell identity are, therefore, important considerations. Orphan nuclear receptor NURR1 (NURR1, also known as NR4A2) is involved in the differentiation of midbrain DA neurons, but also has an important role in the adult brain. Emerging evidence indicates that impaired NURR1 function might contribute to the pathogenesis of PD: NURR1 and its transcriptional targets are downregulated in midbrain DA neurons that express high levels of the disease-causing protein α-synuclein. Clinical and experimental data indicate that disrupted NURR1 function contributes to induction of DA neuron dysfunction, which is seen in early stages of PD. The likely involvement of NURR1 in the development and progression of PD makes this protein a potentially interesting target for therapeutic intervention.

Decressac, M. *et al. Nat. Rev. Neurol.* advance online publication 15 October 2013; doi:10.1038/nrneurol.2013.209

## Introduction

Parkinson disease (PD) is characterized by progressive degeneration of midbrain dopamine (DA) neurons in the substantia nigra (SN) pars compacta and accumulation of intraneuronal Lewy bodies containing misfolded α-synuclein in affected brain areas. Other brain regions are affected in the disease, but important motor symptoms, such as rigidity, bradykinesia, tremor and postural instability, are associated with deficient DA neurotransmission.[1]

Previous studies have demonstrated that striatal DA, and other markers of axonal integrity and synaptic function, are diminished in PD long before cell bodies within the SN actually die.[2] The disease has an early and profound effect on the properties of differentiated DA neurons, which implies that the mechanisms that normally maintain the cellular identity—the phenotype—of midbrain DA neurons are important considerations. During development, the identity of DA neurons is established by the activities of transcription factors that are expressed in the differentiating cells. NURR1 (also known as NR4A2) is one of these developmental transcription factors and is also expressed in mature DA neurons in the adult brain. NURR1 function seems to be perturbed in patients with PD and, in rodents, Nurr1 deficiency is associated with cellular changes that resemble early stages of disease.

In this article, we review studies that have explored the function of NURR1 in mature DA neurons, and describe the emerging evidence for a link between disrupted NURR1 function and PD. We describe the processes that NURR1 regulates, and discuss the ways in which these processes might be associated with PD pathogenesis, and the prospect of using NURR1 as a potential PD drug target.

**Competing interests**
The authors declare no competing interests.

## Role of NURR1 in DA neurons

NURR1 belongs to the family of ligand-activated transcription factors called nuclear receptors. Unlike most other nuclear receptors, NURR1 lacks a hydrophobic pocket for ligand binding and might, therefore, function as a ligand-independent nuclear receptor (Figure 1).[3,4] NURR1 binds to specific DNA binding sites, either as a monomer or homodimer, and can function as a constitutively active transcription factor. However, NURR1 can also form heterodimers with the retinoid X receptor (RXR) that are robustly activated by RXR ligands.[5] NURR1 was first shown to be associated with DA neuron function by its critical involvement in midbrain DA neuron development.[6] *Nurr1* is expressed in the ventral midbrain early in post-mitotic cells (from embryonic day 10.5 in mice) as they begin to express features of DA neurons such as tyrosine hydroxylase (TH) and aromatic amino acid carboxylase (AADC). Importantly, genetic ablation of *Nurr1* in mice revealed that this gene is required for development of midbrain DA neurons.[7] Nurr1-deficient cells initially express some DA neuron markers, although they are unable to innervate their normal forebrain target areas. DA neurons are absent in the SN and the ventral tegmental area in newborn homozygous *Nurr1*-deficient (*Nurr1*$^{-/-}$) mice.[7] By contrast, DA neurons in other parts of the brain develop normally.

That a transcription factor important for developing DA neurons would be interesting from the perspective of PD is not necessarily intuitive. However, *Nurr1* continues to be expressed in postnatal DA neurons,

Wallenberg Neuroscience Centre, Department of Experimental Medical Sciences, Lund University, BMC A11, Lund 22184, Sweden (**M. Decressac, A. Björklund**). Ludwig Institute for Cancer Research, Karolinska Institute, S-171 77 Stockholm, Sweden (**N. Volakakis, T. Perlmann**).

Correspondence to: T. Perlmann thomas.perlmann@licr.ki.se

**Key points**

- NURR1 and other transcription factors that are essential for the development and specification of midbrain dopamine neurons during development continue to have an important role in the adult brain
- Deletion of NURR1 in mature dopamine neurons results in progressive pathology, reduction in dopamine neuron markers and motor impairments—recapitulating early features of Parkinson disease
- Ablation of *Nurr1* in adult rodents results in reduced expression of genes associated with mitochondrial function and oxidative phosphorylation, suggesting a role for Nurr1 in the maintenance of midbrain dopamine neurons
- NURR1 and its transcriptional targets are downregulated in midbrain dopamine neurons that express high levels of the disease-causing protein α-synuclein, as observed in rodent models and patients with Parkinson disease
- α-Synuclein overexpression results in an almost complete blockade of GDNF trophic signalling and failure of GDNF to protect against α-synuclein-induced toxicity in affected neurons
- Reduced NURR1 expression might result in induction of dopamine neuron dysfunction as well progression of degenerative changes, which could make this protein a promising therapeutic target

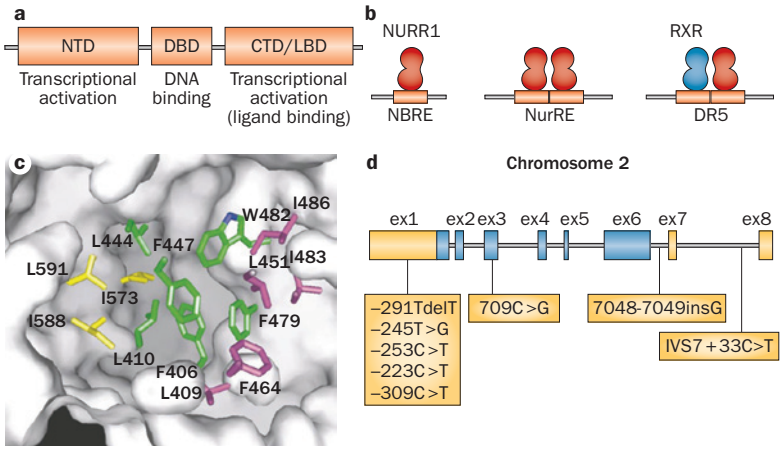


**Figure 1** | Summary of NURR1 functional domains, mode of DNA binding and mutations identified in the human *NURR1* gene (*NR4A2*). **a** | The functional domains of the NURR1 protein. NURR1 has two regions that are important for transcriptional activation—the NTD and the CTD. The protein contains a centrally localized DBD and a domain that is structurally related to nuclear receptor LBD that is localized in the C-terminal part of the protein. **b** | NURR1 can bind to specific DNA-binding sites as a monomer, homodimer or heterodimer with RXR. These heterodimers are efficiently activated by RXR ligands. **c** | Structural analysis has demonstrated that NURR1 does not have a classic nuclear receptor ligand-binding pocket in its putative LBD. Instead, this region is filled with hydrophobic amino acid side chains, leaving little space for ligand binding. **d** | The exon and intron organization of the human *NURR1* gene. Genetic variants that have been associated with PD are indicated. Abbreviations: CTD, C-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain; NTD, N-terminal domain; RXR, retinoid X receptor. Permission for part c obtained from Macmillan Publishers Ltd © Wang, Z. *et al. Nature* **423**, 555–560 (2003).

and accumulating evidence indicates that Nurr1 has an important role in the ongoing maintenance of these cells. DA neurons of heterozygous *Nurr1*-deficient (*Nurr1*$^{+/-}$) mice seem to have an increased susceptibility to toxic stress including conditions that are known to have an effect on DA neuron survival, such as exposure to the mitochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the proteasome inhibitor lactacystin, and metamphetamine.[8–10] Moreover, progressive nigrostriatal dysfunction, a characteristic of PD, has been noted in aged *Nurr1*$^{+/-}$ mice.[11]

The increased vulnerability of DA neurons in heterozygous *Nurr1*$^{+/-}$ mice might reflect a developmental defect of the *Nurr1*± genotype rather than a role for Nurr1 in maintaining the function in mature neurons. However, definitive evidence for a role for Nurr1 in the maintenance and survival of mature DA neurons has been provided by conditional gene targeting of *Nurr1* in mice, both in late-differentiating DA neurons and in the adult brain. Ablation of *Nurr1* in maturing DA neurons has been achieved by crossing conditional *Nurr1* gene targeted (floxed) mice with mice carrying Cre recombinase under control of the dopamine transporter (*DAT*; also known as *SLC6A3*) locus. This late developmental knockout leads to rapid loss of striatal DA, loss of midbrain DA neuron markers, and loss of SN DA neuron cell bodies and striatal innervation.[12] To ablate *Nurr1* in adult DA neurons, *Nurr1* floxed mice were crossed with mice harbouring a *CreERT2* allele under the control of the *DAT* locus, which enables tamoxifen-induced ablation of the *Nurr1* gene in adult brains. These mice exhibit progressive DA neuron pathology associated with a modest reduction of DA neuron markers in ventral midbrain and striatum, reduced striatal DA, and impaired motor behaviour.[13] Interestingly, these mice also show progressive development of TH-positive dystrophic dendrites—a sign of pathology that has been noted in early human PD.[2] Collectively, these results demonstrate that *Nurr1* ablation in adult mice recapitulates early features of PD and support the idea that Nurr1 loss-of-function might contribute to PD.

## Genetic association and postmortem analyses

Mutations that affect NURR1 function could compromise the ability of neurons to exert their normal function and cope with environmental or endogenous stress, thereby triggering pathological changes that lead to PD. Mutations in the *NURR1* gene have not been identified as major genetic risk factors for PD. However, a polymorphism (7048–7049insG) in intron 6 of *NURR1* that potentially affects splicing of the gene has been found at higher frequency in patients with familial and sporadic PD compared with healthy individuals.[14–17] An intron 7 variant (IVS7+33C>T) has also been reported, but only in one patient with PD.[18] Two mutations (–291delT and –245T>G) in the noncoding exon 1 within the 5′ untranslated region of *NURR1* have also been reported in 10 individuals of European descent with familial PD.[19] These variants decrease *NURR1* expression in transfected cell lines and in lymphocytes from affected individuals, implying a hypomorphic effect of the mutated allele. Three other variations in exon 1 (–253C>T, –223C>T and –309C>T) were found in patients with sporadic PD, with the –309C>T mutation resulting in reduced *NURR1* mRNA expression and altered striatal gene expression in brain tissue.[20,21] All of these polymorphisms affect intronic or noncoding parts of the *NURR1* gene. Only one coding missense mutation in exon 3 of *NURR1* (709C>G), which markedly attenuates *NURR1*-induced

transcriptional activation,[22] has been identified in a patient with nonfamilial PD.[23]

The mutations in the *NURR1* locus that are associated with an increased risk of PD have a low prevalence, suggesting that the genetic component of sporadic PD that can be attributed to changes in NURR1 function is quite limited. However, even if *NURR1* is not a major susceptibility locus for PD, other insults that influence *NURR1* expression or function might be involved. Of note, *NURR1* expression is downregulated in post-mortem human brain tissue from neuropathologically verified cases of sporadic PD,[24,25] specifically in SN DA neurons with α-synuclein inclusions.[25] The decline in *NURR1* expression correlates with loss of TH immunofluorescence, which suggests that decreased *NURR1* expression might underlie decreased production of DA, and DA-neuron degeneration.

An age-related decline of the number of *NURR1*-expressing DA neurons, with age-related decreases in both *NURR1* and TH expression within individual neurons, has been noted in the ageing human brain.[26,27] Given that ageing is associated with a decline of DA neurons in the SN[28] and that advancing age is one of the primary risk factors for PD, a role for the age-related decline in *NURR1* expression in the occurrence of PD is a tempting proposal. The fact that, in PD, NURR1 loss is restricted to DA neurons with signs of pathology also seems to link NURR1 to the disease process. However, the correlative nature of the associations between alterations in PD and in the ageing brain should be emphasized, and it remains unclear whether the decrease in *NURR1* expression noted in these studies is causally linked to loss of DA phenotype. Nonetheless, the data from conditional knockout mice do seem to indicate that a decline in *Nurr1* expression could be linked to development and progression of PD pathology and, in particular, in the development of early functional and degenerative changes that are seen in affected DA neurons. Finally, also intriguing to note is the identification of NURR1 as a potential peripheral PD biomarker, as demonstrated by downregulation of *NURR1* expression in peripheral blood lymphocytes from patients with PD, independent of medication, disease severity or duration.[29–31] These interesting observations warrant further exploration in large and stratified cohorts of well-characterized patients, particularly those in early-stage PD.

## Regulation of mitochondrial genes

How would deficient or decreased NURR1 function affect DA neurons in PD? NURR1 is a transcription factor that regulates gene expression in developing and mature DA neurons. It has been linked to the regulation of several genes that encode proteins with functions in DA metabolism, neurotransmission and axonal growth, which supports a role for NURR1 in establishment and maintenance of the DA neurotransmitter phenotype.[7,32–37] Moreover, NURR1 controls important genes that are involved not only in the differentiation, but also the survival, of DA neurons.[34,37–40] These targets include proto-oncogene tyrosine-protein kinase receptor Ret (*Ret*), which encodes the signalling receptor for glial cell line-derived neurotrophic factor (GDNF).[7,41,42] Such a target points to an involvement of NURR1 in regulation of DA neuron survival via maintained neurotrophic signalling (discussed below).

Conditional ablation of the *Nurr1* gene, performed by tamoxifen treatment in adult mice, has enabled the study of dysregulated *Nurr1* target genes in adult DA neurons. Laser-capture microdissection of SN and ventral tegmental area DA neurons was combined with next-generation mRNA sequencing to identify *Nurr1* dysregulated genes 1 week after *Nurr1* ablation.[13] Interestingly, the main functional category of downregulated genes in *Nurr1*-ablated neurons was nuclear-encoded mitochondrial genes, including genes important in oxidative phosphorylation. Strikingly, analysis of large regulatory trends revealed that 90% of the genes that encode proteins involved in oxidative phosphorylation are downregulated in Nurr1-deficient DA neurons, which suggests an important function of Nurr1 in sustaining sufficient respiratory activity in these cells.

Mitochondrial dysfunction has been implicated as a major contributor to PD pathology.[43] Particularly intriguing is the fact that genes encoding proteins involved in oxidative phosphorylation correspond to the functional category of genes that are most dysregulated in remaining DA neurons in PD, as demonstrated by a large meta-analysis of genome-wide gene expression studies.[44] The decreased *NURR1* expression observed in patients with PD might, therefore, influence the level of oxidative phosphorylation, and possibly the respiratory function, in remaining DA neurons. The exact mechanism by which NURR1 affects the expression of mitochondrial genes is not yet understood. NURR1 might functionally interact with other transcription factors that are required for expression of nuclear respiratory genes, such as the nuclear respiratory factors NRF1 and NRF2, or with the transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α), which serves as a master regulator of mitochondrial biogenesis and cellular respiration.[45] Future studies to analyse the level of oxidative phosphorylation in models of *NURR1* ablation and overexpression might clarify these issues.

## Neuroprotection and neuroinflammation

In addition to mitochondrial dysfunction, excitotoxicity and oxidative stress have been implicated in the pathology of many disorders associated with neurodegeneration, including PD.[46,47] Cyclic AMP-responsive element-binding protein (CREB) is a transcription factor that is activated in response to these pathophysiological effectors and regulates a gene expression programme that is essential for neuronal protection. Nurr1 is a mediator of CREB-dependent neuroprotective responses in neurons exposed to excitotoxic and oxidative stress, and is also a regulator of neuroprotective genes.[48] Moreover, synaptic *N*-methyl-D-aspartate (NMDA) receptor activation induces CREB-mediated neuroprotection[49] and leads to upregulation of *Nurr1*.[50–52] NMDA receptor-induced *Nurr1* expression has also been linked to induced expression of the survival-promoting brain-derived neurotrophic factor (BDNF).[53]

The above findings confirm previous observations on the protective effect of increased Nurr1 levels against oxidative stress,[54–56] and indicate that Nurr1 is an essential component of a neuroprotective regulatory mechanism downstream of CREB, and is critical for maintenance of neuronal integrity after insults to the brain. A functional partner of Nurr1 in this adaptive response to neuronal stress could be PGC-1α which, in addition to being a key transcriptional regulator of energy metabolism, also functions as a suppressor of reactive oxygen species in neurons. PGC-1α[44,57,58] is also induced by oxidative stress via CREB and seems to represent a parallel and complementary CREB-dependent regulatory neuroprotective pathway.[48] Evidence suggests that expression of PGC-1α might also be perturbed in patients with PD. Interestingly, PGC-1α is a major regulator of nuclear-encoded mitochondrial genes, providing another functional link between these two proteins. However, PGC-1α has not been reported to be a major co-activator of NURR1, and it remains unclear whether the two transcriptional regulators are mechanistically linked.

Finally, apart from apparently having important roles within DA neurons, NURR1 has been proposed to be part of an anti-inflammatory pathway in microglia and astrocytes that protects DA neurons from inflammation-induced death. Neuroinflammation is associated with activated microglia and increased levels of proinflammatory mediators, and might contribute to PD pathology.[59] Nurr1 is induced in mouse microglia *in vivo* after lipopolysaccharide stereotaxic injection (which generates an animal model of PD).[60] In mice in which *Nurr1* is knocked down in microglia and astrocytes through delivery of lentivirus-mediated short hairpin RNA, lipopolysaccharide injection results in loss of DA neurons in the SN and increased expression of inflammatory mediators, indicating that Nurr1 expressed in glia could act as an inhibitor of inflammatory cytokines secreted by activated microglia.[60]

## A mediator of α-synuclein toxicity

As outlined in the discussion above, NURR1 is essential for maintenance of nigral DA neurons under normal physiological conditions, as well as under conditions of cellular damage and stress. A mechanism whereby NURR1 might be important for DA neuron maintenance is via neurotrophic signalling by regulation of the GDNF receptor Ret. Conditional ablation of *Gdnf*, *Ret* or *Nurr1* in adult mice results in progressive pathological changes that resemble early stages of PD.[13,61,62] These observations support the critical role of the Gdnf–Ret–Nurr1 pathway in preservation of nigral DA neuron integrity and function. Exogenously administered GDNF is a potent pro-survival factor for midbrain DA neurons that can provide neuroprotection against 6-hydroxydopamine and 1-methyl-4-phenylpyridinium (MPP+) toxicity in cell culture[63–65] and in toxin-based rodent and primate models of PD.[66–71] Owing to these promising preclinical results, a number of clinical trials have been undertaken that involve administration of GDNF either into the cerebroventricular system, or directly into the putamen, in patients with advanced PD.[72–74] In an alternative approach, another member of the GDNF family, neurturin, has been expressed in the putamen, or in the putamen and SN, using adeno-associated virus (AAV) vector gene delivery.[75,76] Overall, these trials have given mixed and inconclusive results, although some patients have shown a sustained clinical response (associated with evidence of increased DA activity at the site of delivery as measured by $^{18}$F-DOPA PET, as well as increased TH immunoreactivity in a single postmortem case).[73]

Understanding the basis for the failure of GDNF and neurturin to provide substantial benefits in clinical trials is clearly important. The results from our studies now suggest that α-synuclein-induced toxicity could directly interfere with GDNF signalling in DA neurons in patients with PD. AAV-mediated overexpression of α-synuclein in rats can be used as a tool to induce a progressive PD-like neurodegenerative process in nigral DA neurons. In this model, delivery of GDNF, using a treatment protocol that affords consistent and efficient protection in toxin-based models, failed to protect the nigral DA neurons against α-synuclein-induced toxicity.[77,78] Interestingly, the results from two independent studies indicated that expression of *Nurr1* was markedly reduced as a consequence of α-synuclein toxicity.[79,80] This decrease in *Nurr1* expression was observed in both studies despite differences in experimental design. In contrast to the AAV-mediated α-synuclein-overexpression model, the second model was based on transgenic mouse overexpression of a mutant form of α-synuclein (53A>T). Whereas Nurr1 protein but not mRNA downregulation was noted in the transgenic mouse overexpression model, AAV-mediated α-synuclein transduction caused a marked downregulation of both Nurr1 protein and mRNA. This reduction was observed as early as 2 weeks after vector injection in the AAV α-synuclein overexpression model—at a time point at which α-synuclein is well-expressed but before any major pathological changes are evident.[79] Reductions in expression of Nurr1 target genes, including *Ret*, and blockade of the intracellular response to GDNF (as measured by markers of the GDNF-induced signalling cascade) also accompanied this *Nurr1* downregulation.[79]

Observations of both reduced *Ret* expression and blockade of the GDNF-induced trophic response in DA neurons in *Nurr1* conditional knockout mice supports an important role for Nurr1 in PD pathogenesis. Conversely, forced expression of *Nurr1* restores GDNF signalling in α-synuclein-overexpressing DA neurons, and provides near-complete protection of nigral DA neurons against α-synuclein toxicity, also in the absence of exogenous GDNF.[79] Ret expression also seems to be reduced in patients with PD: levels of Ret immunoreactivity in neuromelanin-positive DA neurons in the SN are reduced in patients with PD compared with age-matched healthy controls.[79]

Several potential explanations for inefficient Ret signalling in models of α-synuclein toxicity exist. However, these results further support the idea that α-synuclein might interfere with GDNF signalling via downregulation of *Nurr1* and its transcriptional target *Ret*, not only in α-synuclein overexpression models, but also in human PD.

Judging from the observations from the AAV α-synuclein model, the magnitude of *Ret* downregulation is probably determined by the level of α-synuclein expression. Increased expression of α-synuclein in nigral DA neurons in PD is likely to depend on a combination of factors, including age[27] and alterations in lysosomal function.[81–83] The increase in α-synuclein, as well as the changes in *Ret* expression, is likely to vary, not only from one DA neuron to another (as is evident from immunostained sections),[25] but between patients. Indeed, from available data,[25] the average cellular *NURR1* expression (which we assume will correlate with *Ret* expression) varies about twofold among patients with PD, from about half of normal to within the range seen in healthy, age-matched controls. Taken together, these observations point to the GDNF–Ret–NURR1 pathway as an interesting target for therapeutic interventions in PD.

## α-Synuclein and NURR1 interactions

The impairment of NURR1 function that is induced by α-synuclein overexpression raises the question of how, and by which molecular mechanisms, α-synuclein affects *NURR1* expression. Interestingly, NURR1 is not the only transcription factor that is affected by α-synuclein overexpression. Other transcription factors that are critical for neuronal survival—myocyte enhancer factor-2D, DNA methyltransferase 1, and the transcription factor EB—are also affected in α-synuclein-based models of PD, as well as in nigral DA neurons in postmortem human PD brain.[84–86] These observations point to a possible action of α-synuclein in the nucleus.

α-Synuclein function has so far been almost exclusively studied in the cytoplasm (where it has a role in synaptic function) but, as its name implies, this protein has also been shown to localize to the cell nucleus in physiological conditions as well as in various *in vitro* and *in vivo* disease models.[79,87–91] However, the influence of nuclear α-synuclein on neurodegenerative changes seen in models of PD and, more specifically, the effect of nuclear α-synuclein on gene expression are poorly understood. Genome-wide-profiling studies have highlighted important molecular pathways involved in PD pathology that are associated with broad transcriptional dysregulation,[92] and α-synuclein could plausibly contribute to this phenomenon. The results from studies in cellular and animal models of PD and Lewy body disease indicate that α-synuclein has an effect on gene expression under pathological conditions, but clear evidence for the involvement of α-synuclein on transcriptional regulation in normal, unperturbed cells is lacking.

As shown in *Drosophila melanogaster*, α-synuclein targeted to the nucleus promotes toxicity, whereas cytoplasmic sequestration is protective.[93] In the nucleus, α-synuclein binds directly to histones, thereby inhibiting acetylation by histone acetyltransferases. The toxicity of α-synuclein can be blocked by administration of histone deacetylase inhibitors, both in cell culture and in transgenic flies.[93] Similar results have been obtained in rodent models of PD, in which overexpression of α-synuclein induces a reduction in histone acetyltransferase p300,

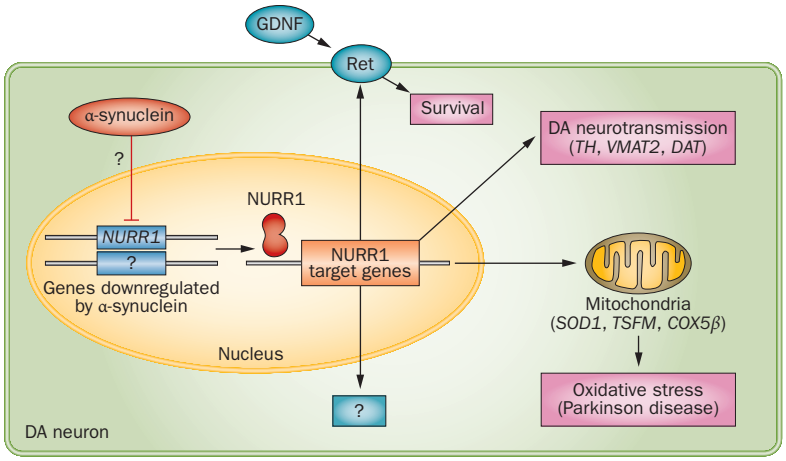


**Figure 2** | The DA neuron functions of NURR1. NURR1 influences expression of several genes involved in neurotransmission and DA metabolism, including *TH*, *VMAT2* and *DAT*. Moreover, NURR1 contributes to survival of DA neurons by regulating expression of *Ret*, the signalling receptor for GDNF and other neurotrophic factors. NURR1 also controls expression of several nuclear-encoded mitochondrial genes such as *SOD1*, *TSFM* and *COX5β*, and seems important for sustained respiratory function. NURR1 is a target of the pathology-promoting α-synuclein, and expression of *NURR1* is reduced by α-synuclein overexpression. Many other genes are probably also downregulated owing to α-synuclein toxicity. α-Synuclein might interfere with GDNF signalling via repression of *NURR1* and its transcriptional target *Ret*. Abbreviations: COX5β, cyclo-oxygenase 5β; DA, dopamine; DAT, dopamine transporter; GDNF, glial-cell-line-derived neurotrophic factor; SOD1, sodium oxide dismutase 1; TH, tyrosine hydroxylase; TSFM, Ts translation elongation factor mitochondrial; VMAT2, vesicular amine transporter 2.

which results in decreased histone acetylation.[79,94] In support of a pathological effect of α-synuclein on gene expression, increased chromatin binding of nuclear α-synuclein has been reported in SN tissue from patients with PD compared with age-matched controls.[95] Furthermore, treatment with histone deacetylase inhibitors can provide protection in *in vitro* and *in vivo* models of α-synuclein toxicity.[93,96]

The above observations provide support for the idea that the changes induced by α-synuclein in the nucleus might drive at least part of the pathological process, and that NURR1 is one of the targets that is directly or indirectly affected. More studies are needed, however, to answer important questions regarding the type, or molecular form, of α-synuclein (monomer or oligomer, phosphorylated or nonphosphorylated) that affects gene expression, the way α-synuclein accesses the nucleus (localization signal or by binding to partners) and the mechanism by which α-synuclein interferes with transcription (direct or indirect binding to transcription factors, or direct binding to DNA).

## Clinical perspective

NURR1 has multiple roles via regulation of genes encoding proteins that are involved in DA neurotransmission (Figure 2). Regulatory targets of NURR1 include CREB-induced survival proteins, nuclear-encoded mitochondrial genes and the neurotrophic signalling receptor Ret. Therefore, drugs designed to activate NURR1 function seem likely to be of therapeutic relevance for

patients with PD. Such drugs might not only be powerful as neuroprotective agents by themselves, but could also be useful as a tool to increase the efficacy of neuroprotective therapies based on GDNF or neurturin delivery. Despite filling of the receptor pocket by hydrophobic side chains (Figure 1), NURR1 might retain the ability to bind small compounds that could be used to modulate its activity. Indeed, although the exact modes of action remain uncertain, several NURR1-activating compounds have been identified.[97–105] RXR could also be a target for modulation of NURR1-regulated processes, as NURR1 can form heterodimers with RXR that are efficiently activated by RXR ligands (Figure 1). However, whether NURR1–RXR heterodimers are associated with any of the important functions mediated by NURR1 in DA neurons remains unclear. Moreover, RXR is a pleiotropic heterodimer partner of several other nuclear receptors, and RXR ligands will presumably regulate genes independently of NURR1 and lead to adverse effects. Alternatively, development of RXR-binding ligands that selectively activate NURR1–RXR heterodimers might be possible.[106,107]

Viral vector delivery of *NURR1* protects DA neurons from α-synuclein-mediated toxicity.[79] Such an intervention—alone or in combination with GDNF or neurturin—is, therefore, an interesting alternative approach that could be considered in future preclinical research. *NURR1* is expressed in cells other than midbrain DA neurons, both inside and outside the brain, so the ability of local vector delivery to target *NURR1* expression specifically to the affected DA neurons is an attractive feature of such gene therapy. In one clinical trial, AAV–neurturin was delivered to patients with PD at two sites in the brain (the putamen and the SN). The outcome of this trial was negative.[108] However, clinical response to this treatment might improve if the nigral injection is replaced or combined with an injection of an AAV–*NURR1* vector.

## Conclusions

The effect of α-synuclein in the pathogenesis of PD has, so far, been focused on its role in the cytoplasm, and the impairment of cellular functions through the production of toxic molecular species and oligomers. Evidence now suggests, however, that α-synuclein enters the nucleus and exerts an effect on the genomic level that results in detrimental changes in the expression of genes involved in maintenance and survival of midbrain DA neurons. The studies reviewed here identify NURR1 as a central player in this genomic cascade, and show that the reduction in NURR1 levels observed in midbrain DA neurons that overexpress α-synuclein, as well as in nigral neurons in human PD, is accompanied by impaired expression of important proteins involved in dopaminergic neurotransmission. For example, a marked reduction in the expression of the GDNF receptor, *Ret*, and an almost complete blockade of GDNF trophic signalling in the affected DA neurons has been reported. We propose that reduced *NURR1* expression, induced by increased cellular levels of α-synuclein, has an important part in induction of DA neuron dysfunction in early stages of PD, and that loss of NURR1 contributes to development and progression of degenerative changes in the affected DA neurons. Together, the clinical and experimental data identify NURR1 as a potential therapeutic target for disease intervention in patients with PD.

### Review criteria

PubMed was searched for peer-reviewed original research articles in English, published up to July 2013, including electronic early-release publications. Search terms included "Parkinson", "Nurr1 or Nr4a2" and "alpha synuclein". The abstracts of retrieved citations were reviewed and prioritized by relevant content. Full articles were obtained and secondary references from these articles were screened for inclusion.


1. Lees, A. J., Hardy, J. & Revesz, T. Parkinson's disease. *Lancet* **373**, 2055–2066 (2009).
2. Cheng, H. C., Ulane, C. M. & Burke, R. E. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann. Neurol.* **67**, 715–725 (2010).
3. Wang, Z. *et al.* Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature* **423**, 555–560 (2003).
4. Baker, K. D. *et al.* The *Drosophila* orphan nuclear receptor DHR38 mediates an atypical ecdysteroid signaling pathway. *Cell* **113**, 731–742 (2003).
5. Perlmann, T. & Jansson, L. A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. *Genes Dev.* **9**, 769–782 (1995).
6. Smidt, M. P. & Burbach, J. P. How to make a mesodiencephalic dopaminergic neuron. *Nat. Rev. Neurosci.* **8**, 21–32 (2007).
7. Zetterstrom, R. H. *et al.* Dopamine neuron agenesis in Nurr1-deficient mice. *Science* **276**, 248–250 (1997).
8. Le, W., Conneely, O. M., He, Y., Jankovic, J. & Appel, S. H. Reduced Nurr1 expression increases the vulnerability of mesencephalic dopamine neurons to MPTP-induced injury. *J. Neurochem.* **73**, 2218–2221 (1999).
9. Pan, T. *et al.* Nurr1 deficiency predisposes to lactacystin-induced dopaminergic neuron injury *in vitro* and *in vivo*. *Brain Res.* **1222**, 222–229 (2008).
10. Luo, Y., Wang, Y., Kuang, S. Y., Chiang, Y. H. & Hoffer, B. Decreased level of Nurr1 in heterozygous young adult mice leads to exacerbated acute and long-term toxicity after repeated methamphetamine exposure. *PLoS ONE* **5**, e15193 (2010).
11. Jiang, C. *et al.* Age-dependent dopaminergic dysfunction in Nurr1 knockout mice. *Exp. Neurol.* **191**, 154–162 (2005).
12. Kadkhodaei, B. *et al.* Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. *J. Neurosci.* **29**, 15923–15932 (2009).
13. Kadkhodaei, B. *et al.* Transcription factor Nurr1 maintains fiber integrity and nuclear-encoded mitochondrial gene expression in dopamine neurons. *Proc. Natl Acad. Sci. USA* **110**, 2360–2365 (2013).
14. Zheng, K., Heydari, B. & Simon, D. K. A common NURR1 polymorphism associated with Parkinson disease and diffuse Lewy body disease. *Arch. Neurol.* **60**, 722–725 (2003).
15. Chen, C. M. *et al.* Nuclear receptor *NR4A2* IVS6 +18insG and brain derived neurotrophic factor (*BDNF*) V66M polymorphisms and risk of Taiwanese Parkinson's disease. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **144B**, 458–462 (2007).
16. Liu, H. *et al.* Genetic analysis of *NR4A2* gene in a large population of Han Chinese patients with Parkinson's disease. *Eur. J. Neurol.* **20**, 584–587 (2013).
17. Wu, Y. *et al.* Association of the polymorphisms in NURR1 gene with Parkinson's disease [Chinese]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* **25**, 693–696 (2008).
18. Tan, E. K. *et al.* Genetic analysis of Nurr1 haplotypes in Parkinson's disease. *Neurosci. Lett.* **347**, 139–142 (2003).
19. Le, W. D. *et al.* Mutations in *NR4A2* associated with familial Parkinson disease. *Nat. Genet.* **33**, 85–89 (2003).
20. Sleiman, P. M. *et al.* Characterisation of a novel *NR4A2* mutation in Parkinson's disease brain. *Neurosci. Lett.* **457**, 75–79 (2009).
21. Hering, R. *et al.* Extended mutation analysis and association studies of *Nurr1* (*NR4A2*) in Parkinson disease. *Neurology* **62**, 1231–1232 (2004).
22. Jacobsen, K. X. *et al.* A *Nurr1* point mutant, implicated in Parkinson's disease, uncouples ERK1/2-dependent regulation of tyrosine

hydroxylase transcription. *Neurobiol. Dis.* **29**, 117–122 (2008).
23. Grimes, D. A. *et al.* Translated mutation in the *Nurr1* gene as a cause for Parkinson's disease. *Mov. Disord.* **21**, 906–909 (2006).
24. Moran, L. B. *et al.* Analysis of alpha-synuclein, dopamine and parkin pathways in neuropathologically confirmed parkinsonian nigra. *Acta Neuropathol.* **113**, 253–263 (2007).
25. Chu, Y. *et al.* Nurr1 in Parkinson's disease and related disorders. *J. Comp. Neurol.* **494**, 495–514 (2006).
26. Chu, Y., Kompoliti, K., Cochran, E. J., Mufson, E. J. & Kordower, J. H. Age-related decreases in Nurr1 immunoreactivity in the human substantia nigra. *J. Comp. Neurol.* **450**, 203–214 (2002).
27. Chu, Y. P. & Kordower, J. H. Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? *Neurobiol. Dis.* **25**, 134–149 (2007).
28. Fearnley, J. M. & Lees, A. J. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* **114**, 2283–2301 (1991).
29. Le, W. *et al.* Decreased *NURR1* gene expression in patients with Parkinson's disease. *J. Neurol. Sci.* **273**, 29–33 (2008).
30. Liu, H. *et al.* Decreased *NURR1* and *PITX3* gene expression in Chinese patients with Parkinson's disease. *Eur. J. Neurol.* **19**, 870–875 (2012).
31. Pan, T. H., Xie, W. J., Jankovic, J. & Le, W. D. Decreased Nurr1 mRNA in peripheral blood lymphocytes in Parkinson's disease. *Neurology* **62**, A108–A108 (2004).
32. Saucedo-Cardenas, O. *et al.* Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc. Natl Acad. Sci. USA* **95**, 4013–4018 (1998).
33. Eells, J. B., Misler, J. A. & Nikodem, V. M. Reduced tyrosine hydroxylase and GTP cyclohydrolase mRNA expression, tyrosine hydroxylase activity, and associated neurochemical alterations in Nurr1-null heterozygous mice. *Brain Res. Bull.* **70**, 186–195 (2006).
34. Jacobs, F. M. *et al.* Identification of *Dlk1*, *Ptpru* and *Klhl1* as novel Nurr1 target genes in meso-diencephalic dopamine neurons. *Development* **136**, 2363–2373 (2009).
35. Hermanson, E. *et al.* Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. *Exp. Cell Res.* **288**, 324–334 (2003).
36. Smits, S. M., Ponnio, T., Conneely, O. M., Burbach, J. P. & Smidt, M. P. Involvement of Nurr1 in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. *Eur. J. Neurosci.* **18**, 1731–1738 (2003).
37. Gil, M. *et al.* Regulation of GTP cyclohydrolase I expression by orphan receptor Nurr1 in cell culture and *in vivo*. *J. Neurochem.* **101**, 142–150 (2007).
38. Luo, Y. *et al.* VIP is a transcriptional target of Nurr1 in dopaminergic cells. *Exp. Neurol.* **203**, 221–232 (2007).
39. Heng, X. *et al. Nurr1* regulates *Top IIβ* and functions in axon genesis of mesencephalic dopaminergic neurons. *Mol. Neurodegener.* **7**, 4 (2012).
40. Joseph, B. *et al.* $p57^{Kip2}$ cooperates with Nurr1 in developing dopamine cells. *Proc. Natl Acad. Sci. USA* **100**, 15619–15624 (2003).
41. Galleguillos, D. *et al.* Nurr1 regulates RET expression in dopamine neurons of adult rat midbrain. *J. Neurochem.* **114**, 1158–1167 (2010).
42. Wallen, A. A. *et al.* Orphan nuclear receptor Nurr1 is essential for Ret expression in midbrain dopamine neurons and in the brain stem. *Mol. Cell. Neurosci.* **18**, 649–663 (2001).
43. Exner, N., Lutz, A. K., Haass, C. & Winklhofer, K. F. Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. *EMBO J.* **31**, 3038–3062 (2012).
44. Zheng, B. *et al.* PGC-1α, a potential therapeutic target for early intervention in Parkinson's disease. *Sci. Transl. Med.* **2**, 52ra73 (2010).
45. Puigserver, P. & Spiegelman, B. M. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator. *Endocr. Rev.* **24**, 78–90 (2003).
46. Doble, A. The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol. Ther.* **81**, 163–221 (1999).
47. Halliwell, B. Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* **97**, 1634–1658 (2006).
48. Volakakis, N. *et al.* NR4A orphan nuclear receptors as mediators of CREB-dependent neuroprotection. *Proc. Natl Acad. Sci. USA* **107**, 12317–12322 (2010).
49. Zhang, S. J. *et al.* Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. *Neuron* **53**, 549–562 (2007).
50. Zhang, S. J. *et al.* Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity. *PLoS Genet.* **5**, e1000604 (2009).
51. Xiang, G. *et al.* Identification of activity-dependent gene expression profiles reveals specific subsets of genes induced by different routes of $Ca^{2+}$ entry in cultured rat cortical neurons. *J. Cell. Physiol.* **212**, 126–136 (2007).
52. Pegoraro, S. *et al.* Sequential steps underlying neuronal plasticity induced by a transient exposure to gabazine. *J. Cell. Physiol.* **222**, 713–728 (2010).
53. Barneda-Zahonero, B. *et al.* Nurr1 protein is required for *N*-methyl-D-aspartic acid (NMDA) receptor-mediated neuronal survival. *J. Biol. Chem.* **287**, 11351–11362 (2012).
54. Lee, M. A. *et al.* Overexpression of midbrain-specific transcription factor Nurr1 modifies susceptibility of mouse neural stem cells to neurotoxins. *Neurosci. Lett.* **333**, 74–78 (2002).
55. Sousa, K. M. *et al.* Microarray analyses support a role for Nurr1 in resistance to oxidative stress and neuronal differentiation in neural stem cells. *Stem Cells* **25**, 511–519 (2007).
56. Jo, A. Y. *et al.* Generation of dopamine neurons with improved cell survival and phenotype maintenance using a degradation-resistant nurr1 mutant. *Stem Cells* **27**, 2238–2246 (2009).
57. Shin, J. H. *et al.* PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. *Cell* **144**, 689–702 (2011).
58. Pacelli, C. *et al.* Mitochondrial defect and PGC-1α dysfunction in parkin-associated familial Parkinson's disease. *Biochim. Biophys. Acta* **1812**, 1041–1053 (2011).
59. Deleidi, M. & Gasser, T. The role of inflammation in sporadic and familial Parkinson's disease. *Cell. Mol. Life Sci.* http://dx.doi.org/10.1007/s00018-013-1352-y.
60. Saijo, K. *et al.* A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* **137**, 47–59 (2009).
61. Kramer, E. R. *et al.* Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system. *PLoS Biol.* **5**, e39 (2007).
62. Pascual, A. *et al.* Absolute requirement of GDNF for adult catecholaminergic neuron survival. *Nat. Neurosci.* **11**, 755–761 (2008).
63. Eggert, K. *et al.* Glial cell line-derived neurotrophic factor protects dopaminergic neurons from 6-hydroxydopamine toxicity *in vitro*. *Neurosci. Lett.* **269**, 178–182 (1999).
64. Hou, J. G., Lin, L. F. & Mytilineou, C. Glial cell line-derived neurotrophic factor exerts neurotrophic effects on dopaminergic neurons *in vitro* and promotes their survival and regrowth after damage by 1-methyl-4-phenylpyridinium. *J. Neurochem.* **66**, 74–82 (1996).
65. Lin, L. F., Doherty, D. H., Lile, J. D., Bektesh, S. & Collins, F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* **260**, 1130–1132 (1993).
66. Choi-Lundberg, D. L. *et al.* Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* **275**, 838–841 (1997).
67. Gash, D. M. *et al.* Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* **380**, 252–255 (1996).
68. Grondin, R. *et al.* Chronic, controlled GDNF infusion promotes structural and functional recovery in advanced parkinsonian monkeys. *Brain* **125**, 2191–2201 (2002).
69. Kirik, D., Rosenblad, C., Bjorklund, A. & Mandel, R. J. Long-term rAAV-mediated gene transfer of GDNF in the rat Parkinson's model: intrastriatal but not intranigral transduction promotes functional regeneration in the lesioned nigrostriatal system. *J. Neurosci.* **20**, 4686–4700 (2000).
70. Sauer, H., Rosenblad, C. & Bjorklund, A. Glial cell line-derived neurotrophic factor but not transforming growth factor beta 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc. Natl Acad. Sci. USA* **92**, 8935–8939 (1995).
71. Tomac, A. *et al.* Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*. *Nature* **373**, 335–339 (1995).
72. Lang, A. E. *et al.* Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann. Neurol.* **59**, 459–466 (2006).
73. Gill, S. S. *et al.* Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat. Med.* **9**, 589–595 (2003).
74. Nutt, J. G. *et al.* Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* **60**, 69–73 (2003).
75. Bartus, R. T. *et al.* Bioactivity of AAV2-neurturin gene therapy (CERE-120): differences between Parkinson's disease and nonhuman primate brains. *Mov. Disord.* **26**, 27–36 (2011).
76. Marks, W. J. Jr *et al.* Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol.* **9**, 1164–1172 (2010).
77. Decressac, M. *et al.* GDNF fails to exert neuroprotection in a rat α-synuclein model of Parkinson's disease. *Brain* **134**, 2302–2311 (2011).
78. Lo Bianco, C., Deglon, N., Pralong, W. & Aebischer, P. Lentiviral nigral delivery of GDNF does not prevent neurodegeneration in a genetic rat model of Parkinson's disease. *Neurobiol. Dis.* **17**, 283–289 (2004).
79. Decressac, M. *et al.* α-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. *Sci. Transl. Med.* **163**, 163ra156 (2012).
80. Lin, X. *et al.* Conditional expression of Parkinson's disease-related mutant α-synuclein

in the midbrain dopaminergic neurons causes progressive neurodegeneration and degradation of transcription factor nuclear receptor related 1. *J. Neurosci.* **32**, 9248–9264 (2012).
81. Chu, Y., Dodiya, H., Aebischer, P., Olanow, C. W. & Kordower, J. H. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. *Neurobiol. Dis.* **35**, 385–398 (2009).
82. Crews, L. *et al.* Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of α-synucleinopathy. *PLoS ONE* **5**, e9313 (2010).
83. Dehay, B. *et al.* Pathogenic lysosomal depletion in Parkinson's disease. *J. Neurosci.* **30**, 12535–12544 (2010).
84. Chu, Y., Mickiewicz, A. L. & Kordower, J. H. α-Synuclein aggregation reduces nigral myocyte enhancer factor-2D in idiopathic and experimental Parkinson's disease. *Neurobiol. Dis.* **41**, 71–82 (2011).
85. Decressac, M. *et al.* TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *Proc. Natl Acad. Sci. USA* **110**, E1817–E1826 (2013).
86. Desplats, P. *et al.* α-Synuclein sequesters Dnmt1 from the nucleus: a novel mechanism for epigenetic alterations in Lewy body diseases. *J. Biol. Chem.* **286**, 9031–9037 (2011).
87. Azeredo da Silveira, S. *et al.* Phosphorylation does not prompt, nor prevent, the formation of α-synuclein toxic species in a rat model of Parkinson's disease. *Hum. Mol. Genet.* **18**, 872–887 (2009).
88. Masliah, E. *et al.* Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders. *Science* **287**, 1265–1269 (2000).
89. McLean, P. J., Ribich, S. & Hyman, B. T. Subcellular localization of α-synuclein in primary neuronal cultures: effect of missense mutations. *J. Neural Transm. Suppl.* **2000**, 53–63 (2000).
90. Specht, C. G. *et al.* Subcellular localisation of recombinant α- and γ-synuclein. *Mol. Cell. Neurosci.* **28**, 326–334 (2005).
91. Takahashi, M. *et al.* Phosphorylation of α-synuclein characteristic of synucleinopathy lesions is recapitulated in α-synuclein transgenic *Drosophila*. *Neurosci. Lett.* **336**, 155–158 (2003).
92. Cooper-Knock, J. *et al.* Gene expression profiling in human neurodegenerative disease. *Nat. Rev. Neurol.* **8**, 518–530 (2012).
93. Kontopoulos, E., Parvin, J. D. & Feany, M. B. α-Synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. *Hum. Mol. Genet.* **15**, 3012–3023 (2006).
94. Jin, H. *et al.* α-Synuclein negatively regulates protein kinase Cδ expression to suppress apoptosis in dopaminergic neurons by reducing p300 histone acetyltransferase activity. *J. Neurosci.* **31**, 2035–2051 (2011).
95. Siddiqui, A. *et al.* Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson's disease. *Free Radic. Biol. Med.* **53**, 993–1003 (2012).
96. Outeiro, T. F. *et al.* Sirtuin 2 inhibitors rescue α-synuclein-mediated toxicity in models of Parkinson's disease. *Science* **317**, 516–519 (2007).
97. Chintharlapalli, S. *et al.* Activation of Nur77 by selected 1,1-Bis(3′-indolyl)-1-(*p*-substituted phenyl)methanes induces apoptosis through nuclear pathways. *J. Biol. Chem.* **280**, 24903–24914 (2005).
98. Dubois, C., Hengerer, B. & Mattes, H. Identification of a potent agonist of the orphan nuclear receptor Nurr1. *ChemMedChem* **1**, 955–958 (2006).
99. Hintermann, S. *et al.* Identification of a series of highly potent activators of the Nurr1 signaling pathway. *Bioorg. Med. Chem. Lett.* **17**, 193–196 (2007).
100. Inamoto, T. *et al.* 1,1-Bis(3′-indolyl)-1-(*p*-chlorophenyl)methane activates the orphan nuclear receptor Nurr1 and inhibits bladder cancer growth. *Mol. Cancer Ther.* **7**, 3825–3833 (2008).
101. Li, X., Lee, S. O. & Safe, S. Structure-dependent activation of NR4A2 (Nurr1) by 1,1-bis (3′-indolyl)-1-(aromatic)methane analogs in pancreatic cancer cells. *Biochem. Pharmacol.* **83**, 1445–1455 (2012).
102. Ordentlich, P., Yan, Y., Zhou, S. & Heyman, R. A. Identification of the antineoplastic agent 6-mercaptopurine as an activator of the orphan nuclear hormone receptor Nurr1. *J. Biol. Chem.* **278**, 24791–24799 (2003).
103. Poppe, L. *et al.* Discovery of ligands for Nurr1 by combined use of NMR screening with different isotopic and spin-labeling strategies. *J. Biomol. Screen* **12**, 301–311 (2007).
104. Wansa, K. D., Harris, J. M., Yan, G., Ordentlich, P. & Muscat, G. E. The AF-1 domain of the orphan nuclear receptor NOR-1 mediates trans-activation, coactivator recruitment, and activation by the purine anti-metabolite 6-mercaptopurine. *J. Biol. Chem.* **278**, 24776–24790 (2003).
105. Zhang, Z. *et al.* Anti-parkinsonian effects of Nurr1 activator in ubiquitin-proteasome system impairment induced animal model of Parkinson's disease. *CNS Neurol. Disord. Drug Targets* **11**, 768–773 (2012).
106. Wallen-Mackenzie, A. *et al.* Nurr1-RXR heterodimers mediate RXR ligand-induced signaling in neuronal cells. *Genes Dev.* **17**, 3036–3047 (2003).
107. Morita, K. *et al.* Selective allosteric ligand activation of the retinoid X receptor heterodimers of NGFI-B and Nurr1. *Biochem. Pharmacol.* **71**, 98–107 (2005).
108. Bartus, R. T. *et al.* Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. *Neurology* **80**, 1698–1701 (2013).

**Acknowledgements**
We thank members in our laboratories for valuable discussions. This work was supported by grants from the Swedish Research Council via a Linnaeus grant (A. Björklund & T. Perlmann), the Swedish Strategic Research Foundation (A. Björklund & T. Perlmann) and the Michael J. Fox Foundation (A. Björklund & T. Perlmann). We apologize to the authors of the many interesting studies that could not be included in this Review owing to space constraints.

**Author contributions**
All authors researched data for the article, provided substantial discussion of content, contributed to writing the article, and review and/or editing of the manuscript before submission.