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Review

# Is activation of GDNF/RET signaling the answer for successful treatment of Parkinson's disease? A discussion of data from the culture dish to the clinic

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## From the Contents



## Abstract

The neurotrophic signaling of glial cell line-derived neurotrophic factor (GDNF) with its canonical receptor, the receptor tyrosine kinase RET, coupled together with the GDNF family receptor alpha 1 is important for dopaminergic neuron survival and physiology in cell culture experiments and animal models. This prompted the idea to try GDNF/RET signaling as a therapeutic approach to treat Parkinson's disease with the hallmark of dopaminergic cell death in the substantia nigra of the midbrain. Despite several clinical trials with GDNF in Parkinson's disease patients, which mainly focused on optimizing the GDNF delivery technique, benefits were only seen in a few patients. In general, the endpoints did not show significant improvements. This suggests that it will be helpful to learn more about the basic biology of this fascinating but complicated GDNF/RET signaling system in the dopaminergic midbrain and about recent developments in the field to facilitate its use in the clinic. Here we will refer to the latest publications and point out important open questions in the field.

**Key Words:** α-synuclein; clinical trials; dopaminergic neurons; glial cell line-derived neurotrophic factor; GFRα1; gut-brain axis; Nedd4; Parkin; Parkinson's disease; RET

## Introduction

The receptor tyrosine kinase rearranged during transfection (RET) can signal via several pathways to exert vital functions in neurons. The most widely studied ligand for RET is the glial cell line-derived neurotrophic factor (GDNF), which signals through binding the glycosylphosphatidylinositol (GPI)-linked GDNF family receptor alpha 1 (GFRα1) and to some extent also GDNF family receptor alpha 2 (GFRα2), which in turn binds RET to activate its intracellular tyrosine kinase activity (**Figure 1**). RET is responsible for activating downstream signaling cascades which can promote neurite growth, cell differentiation, and survival, as well as synaptic plasticity (Enterria-Morales et al., 2020). Whilst GDNF-RET signaling has been widely studied for decades, as well as being the target of several clinical trials attempting to treat Parkinson's disease (PD), the full extent of GDNF-RET's physiological functions in the midbrain dopaminergic system, which is impaired in PD, remains unclear.

## Search Strategy

We searched all literature available till spring 2021 by using the terms in the Title, Abstract, and Keywords on PubMed and Google and retrieved further articles by citation tracking.

## *In Vivo* GDNF/RET Signaling in the Dopaminergic System and Beyond

In a recent special issue on neurotrophic factors, Conway et al. (2020) discuss the *in vivo* function of GDNF-RET signaling in midbrain dopaminergic neurons. The phenotypes of mice with GDNF and its canonical receptor RET knocked out are mentioned and data on the crosstalk of RET with genes known to be mutated in familial forms of PD, including *PARK1/SNCA* (α-synuclein), *PARK7/DJ1*, *PARK2/Parkin* (parkin) and *PARK6/PINK1* are summarized (Kramer et al., 2007; Kramer and Liss, 2015; Meka et al., 2015). RET deficient mice specifically lose dopaminergic neurons in the substantia nigra during aging (Kramer et al., 2007), whilst more recent data support the notion that there is no or only a very mild defect on the maintenance of midbrain dopaminergic neurons in GDNF deficient mice (Kopra et al., 2015; Enterria-Morales et al., 2020). However, GDNF deficiency in mice might lead to a reduced amphetamine-induced locomotor response and striatal dopamine efflux (Kopra et al., 2017). GDNF seems therefore not essential for the development and maintenance of the midbrain dopaminergic system but is important for its normal physiological function, as well as remaining a viable therapeutic. This suggests that RET might additionally be activated in the dopaminergic system independently of GDNF by alternative signaling events, such as cross-activation by other receptor tyrosine kinases (Volinsky and Kholodenko, 2013). Further research is needed to confirm this hypothesis. However, GDNF/RET signaling might be limited during mouse development, as a constitutively active RET mutation, MEN2B, and GDNF overexpression on its native locus increases the number of adult dopamine neurons in the substantia nigra pars compacta and the number of dopaminergic terminals in the dorsal striatum (Mijatovic et al., 2007; Kumar et al., 2015). More detailed analysis of when this phenotype appears and the mechanism at play is still required in these mice.

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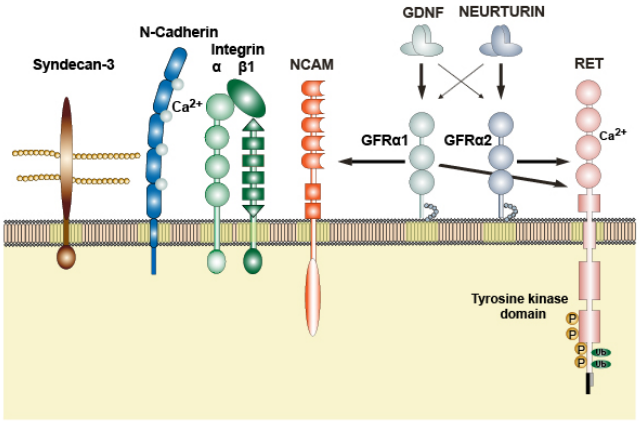


**Figure 1** | **GDNF family of ligands and the different GDNF receptors.**
In the dopaminergic system only two out of the four GDNF-family members are of interest, the glial cell line-derived neurotrophic factor (GDNF) and neurturin. These ligands form homodimers that undergo high-affinity binding with one of the four GDNF family receptor α members (GFRα1–4) with only GFRα1 being expressed in the dopaminergic system. The receptor-ligand complexes may then interact with RET, GDNF's canonical receptor, or also activate alternative GDNF receptors including syndecan-3, N-cadherin, integrin α and β1 and the neural cell adhesion molecule (NCAM). RET's intracellular domain can be subject to post-translational modifications, including phosphorylation at a number of tyrosine and serine domains and ubiquitination. RET51 (long) and RET9 (short) isoforms, which differ only in their cytoplasmic domains, may be subject to different post-translational modifications. The phosphorylation sites on RET provide docking sites for adaptor proteins, resulting in differing downstream signaling cascades.

The GDNF receptor GFRα1 has not yet been specifically deleted in dopaminergic neurons in the mouse and therefore the phenotype of adult GFRα1 deficient mice has not yet been reported, despite the creation of a floxed GFRα1 allele (Uesaka et al., 2007). Studies in adult GFRα1 global knockout mice are not possible, as these die shortly after birth due to renal agenesis, as do GDNF global knockout mice (Kramer and Liss, 2015). Only heterozygous GFRα1 knockout mice have been generated showing a mild age-related inflammation, reduction in the number of tyrosine hydroxylase-positive cells in the substantia nigra, reduced striatal dopamine and tyrosine hydroxylase staining and a reduced motor function also after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment (Boger et al., 2008; Zaman et al., 2008). Without the data from the conditional GFRα1 deficient mice in dopaminergic neurons, the picture of GDNF/RET/GFRα1 signaling in the dopaminergic system remains incomplete. Further important information might be gained from investigating GDNF/RET/GFRα1 signaling in human dopaminergic neurons, which so far remains a largely unexplored area of research.

Also, the role of alternative GDNF receptors such as syndecan-3, N-cadherin, integrins and NCAM in the dopaminergic system has not been carefully studied and requires more work (**Figure 1**). Despite recent data showing no effect of exogenous GDNF or RET agonists in RET deficient mice and cells from RET deficient mice, respectively (Drinkut et al., 2016; Mahato et al., 2020), this does not rule out a function of these alternative GDNF receptors in the dopaminergic system perhaps even without GDNF, as cell adhesion factors and in different signaling cascades.

The protein parkin appears to be an interesting crosstalk partner of GDNF/RET (Meka et al., 2015). Parkin is an E3 ubiquitin ligase that appears to mono- or poly-ubiquitinate proteins on the outer mitochondrial membrane as a result of cellular insults (Yoshii et al., 2011). In turn, this mediates the removal of damaged mitochondria by autophagy. Mice deficient for both parkin and RET exhibited an accelerated loss of both dopaminergic neurons and axons when compared with mice deficient for parkin or RET only, which showed no and moderate degeneration, respectively (Meka et al., 2015). Parkin overexpression provided a neuroprotective effect on the midbrain dopaminergic system of aged RET-deficient mice, which is consistent with a system of tight parkin/RET crosstalk (Meka et al., 2015). RET and parkin signaling proved to be important for mitochondrial integrity through activation of the pro-survival NF-κB pathway, which was mediated by RET through PI3K signaling (Meka et al., 2015). These data are encouraging, as GDNF/RET signaling may be able to target the often critically impaired mitochondrial function in both familial and sporadic forms of PD. RET has not been found to be a substrate of parkin, however in cell culture experiments other E3 ubiquitin ligases, including the E3 ligase Casitas B-lineage Lymphoma and the neural precursor cell-expressed developmentally downregulated protein 4 (Nedd4) have been shown to target the long and the short isoforms of RET (RET9), which differ only in their cytoplasmic domains, respectively (Ishiguro et al., 1999; Hyndman et al., 2017) (**Figure 1**). As a part of the Nedd4/GRB10/SHANK2 complex, Nedd4 localizes to clathrin-coated pits and may play an important role in RET9 ubiquitination, endocytosis, and degradation (Hyndman et al., 2017). Nedd4 may also ubiquitinate α-synuclein, resulting in its clearance via the endosome-lysosome pathway (Tofaris et al., 2011). Therefore, Nedd4 expression in midbrain dopaminergic neurons might be able to protect from α-synuclein toxicity and downregulate RET neurotrophic signaling simultaneously. It would be of great interest to study these possible functions of Nedd4 in more detail *in vivo* concerning stimulating RET and/or Nedd4 function in PD patients to protect midbrain dopaminergic neurons.

In addition, the review by Conway et al. (2020) comprehensively discusses *in vivo* mRNA and protein expression data for GDNF, GFRα1, and RET in humans and rodents during development, adulthood, and aging. Within the midbrain, only dopaminergic neurons appear to express RET and GFRα1 (Kramer and Liss, 2015), with cells in the striatum only expressing GFRα1 (Kramer et al., 2007). The consensus in the literature is that there is no alteration in GFRα1 and RET expression in aging mammals (Kramer et al., 2007). Regarding PD patients with an accumulation of α-synuclein, an open discussion remains as to whether RET protein level may be reduced. Viral overexpression of α-synuclein in the rat substantia nigra has been found to lead to neurodegeneration of dopaminergic neurons that cannot be prevented by GDNF treatment (Lo Bianco et al., 2004; Decressac et al., 2011), which is thought to be mediated through transcriptional and translational downregulation of the transcription factor nuclear receptor related 1 (Nurr1) and its target gene RET (Wallen et al., 2001; Decressac et al., 2012). It seems that high viral overexpression of α-synuclein levels can cause an interruption of GDNF/RET signaling in rats which might not be of physiological relevance nor recapitulate the situation in humans, where a triplication of the α-synuclein locus leads to only double the amount of soluble α-synuclein but still an early-onset form of PD (Olgiati et al., 2015; Albert et al., 2017; Duffy et al., 2018; Polinski et al., 2018). α-Synuclein mRNA expression seems to not be upregulated in PD patients (Su et al., 2017) and can even be downregulated (Kingsbury et al., 2004), with no alteration of Nurr1 or growth factor receptors observed in PD patients either (Su et al., 2017). It has also been shown that no (Backman et al., 2006; Su et al., 2017) or very little (Decressac et al., 2012) reduction in RET is observed in the dopaminergic system of PD patients. It remains an open question how much RET is needed to mediate GDNF's beneficial effect on dopaminergic neurons. Recently it has been reported that GDNF overexpression may protect from α-synuclein triggered dopamine neuron death in cell culture and mice (Chmielarz et al., 2020). Further research is needed to clarify the crosstalk of α-synuclein and GDNF/RET signaling in the midbrain dopaminergic system.

It has become more widely accepted that PD starts outside of the brain most likely in the gut, where the first α-synuclein aggregates are found in the enteric nervous system and neurons seem to die which correlates with the early PD non-motor symptoms such as constipation (Siddiqui et al., 2002; Braak et al., 2006; Pedrosa Carrasco et al., 2018). This α-synuclein pathology seems to spread along the gut-brain axis through the dorsal motor nucleus of the vagus nerve into the brainstem leading to rapid eye movement (REM) sleep behavior disorder, depression and finally leading to the typical PD motor symptoms via the death of midbrain dopaminergic neurons of the substantia nigra (Greene, 2014; Braak and Del Tredici, 2017; Schaeffer et al., 2020). Interestingly, the neurons of the gut-brain axis also express RET (Nosrat et al., 1997; Wallen et al., 2001; Barlow et al., 2003). The RET receptor mediates the development of enteric nervous system neurons but RET's physiological function in the adult enteric nervous system is not well understood (Taraviras et al., 1999). It remains an interesting question what the physiological function of GDNF/RET signaling could be in these different neurons along the gut-brain axis and how far crosstalk of α-synuclein and GDNF/RET signaling takes place here, too.

## Glial Cell Line-Derived Neurotrophic Factor Clinical Trials

Despite these encouraging preclinical data, the results from GDNF clinical trials on PD patients are still inconclusive (Barker et al., 2020; Manfredsson et al., 2020; Sidorova and Saarma, 2020). The first clinical trial saw intraventricular GDNF administration failing to improve motor symptoms in PD patients due to its inability to cross cell barriers, thus not reaching target neurons. Patients in this trial also reported suffering adverse effects including nausea, paraesthesia, weight loss, and anorexia (Nutt et al., 2003). Following an improvement of the knowledge of GDNF's biodistribution features, two small, phase I, open-label trials were embarked upon. These trials employed an intraputaminal delivery of GDNF, which increased $^{18}$F-fluoro-dihydroxyphenylalanine ($^{18}$F-DOPA) uptake on PET scans by 19%, with an absence of major side effects (Gill et al., 2003; Slevin et al., 2005). These effects persisted for several months after the end of GDNF treatment (Slevin et al., 2007). A phase II, placebo-controlled trial followed this in an attempt to replicate the earlier observations but it did not meet its primary endpoints (Lang et al., 2006). Another more recent phase II trial also failed to reach its primary endpoints, however, all patients in the GDNF-treated group had significantly increased $^{18}$F-DOPA uptake on PET scans; when *post hoc* analyses were performed, 43% of these patients had at least a 10 point Unified Parkinson's Disease Rating Scale increase, with no increase in the control group (Whone et al., 2019a, b). Ninety-five percent of these patients had at least one clinically significant outcome measure 80 weeks after administration ended (Whone et al., 2019a, b). In a recent workshop attended by many working on these clinical trials, it was agreed that throughout these trials that a statistical significance is shown in some but not all patients, resulting in the overall trials not meeting their primary endpoint (Barker et al., 2020). The results of the GDNF clinical trials and the influence of placebo and nocebo effect on these studies have also recently been reviewed (Gash et al., 2020). Restoration of motor function was often observed in patients receiving both GDNF and placebo treatments (Gill et al., 2003). One of the factors responsible for this effect may be that many patients are subject to long-term distress as a result of PD. When these patients take positive action by agreeing to participate in a clinical trial, with a positive outcome expected, a more positive outlook develops with time, thus creating a strong placebo effect. This placebo effect might be mediated by increased release of the neurotransmitter dopamine in the brain, which is reduced in PD. The nocebo effect is a term describing here the worsening of symptoms and clinical features in patients with progressive and currently incurable PD due to their own negative perceptions and expectations.

There was great promise for the GDNF clinical trials from the beginning, however, technical problems with the production and delivery of GDNF, as well as the selection of the patients still require optimization (Gill et al., 2003). One issue is that GDNF therapy is only viable for PD patients with sufficient midbrain dopaminergic neurons remaining, which are the only cells in the midbrain expressing the GDNF receptor RET (Kramer and Liss, 2015; Quintino et al., 2019). Therefore, the ideal candidate for GDNF therapy is a younger, early-stage, sporadic PD patient with many dopaminergic neurons left (Barker et al., 2020). This is however a patient that might benefit still for many years from symptomatic treatment. Another issue is that we are still not fully certain as to how GDNF/RET signaling results in maintenance, neuroprotection, and regeneration of midbrain dopaminergic neurons. A difficult issue remains the delivery of GDNF and its close family member neurturin since they do not pass the blood-brain barrier and there is very little or no transport to the substantia nigra if injected in the striatum (Bartus et al., 2015). Intracranial surgery for delivery causes a great burden on PD patients and there have been patients in multiple trials who have developed issues with their cannulae but the Renishaw and Clearpoint systems seem to improve the intracranial delivery with each trial. Other delivery methods including intranasal administration and transient disruption of the blood-brain barrier are also being tested and may hold promise, however, more study is required here (Hernando et al., 2018; Li et al., 2018). In early trials, it is thought that low dose or biological activity of particular GDNF batches which were not sufficient to provide clinical benefit may have caused a variation in results (Kirkeby and Barker, 2019). Interestingly, neurotrophic factors often require only a short term, pulsatile interaction with their receptors to activate the signaling required for neuronal survival, which can persist for days or months afterwards (Sidorova and Saarma, 2020). Overactivation of neurotrophic receptors due to prolonged interaction with their ligand may be detrimental to the neurons due to some negative feedback loops which serve to limit uncontrolled propagation or multiplication of these signals (Lake et al., 2016). Additionally, very high concentrations of growth factors, which produce biphasic response curves in clinical trials, may inhibit the formation of oligomeric signaling complexes required for function because each monomer of the receptor is bound to a ligand (Schlee et al., 2006). For example, GDNF overexpression in rats was shown to downregulate the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase, which is indicative of reduced dopamine syntheses and dopaminergic function (Georgievska et al., 2004). Therefore, the beneficial amount of GDNF to be delivered to patients may be in a narrow range, too much or too little might result in a negative outcome. It was however recently shown in aged mice that a two-fold increase in endogenous GDNF levels enhances dopaminergic function and appears to be safe (Turconi et al., 2020). Despite this, it seems worth exploring alternative delivery methods such as small RET agonistic drugs or gene therapy approaches.

## Small Molecule RET Agonists and Altered RET Ligands – A New Hope?

Since GDNF does not cross the blood-brain barrier and needs to be supplied directly into the brain when used as a PD drug, there are great efforts to develop small RET/GFRα1 activator molecules or GDNF mimetics that do pass the blood-brain barrier (Mahato and Sidorova, 2020). As only very small amounts of neurotrophic factors are required, small quantities of well-designed molecules might yield clinically significant

results, providing that they activate the correct downstream signaling cascades. Examples of such drugs are DNSP-11, XIB4035, Q525, compound 8, BT13 or BT44, which are currently being tested in different *in vivo* and *in vitro* models of PD (Tokugawa et al., 2003; Stenslik et al., 2015; Ivanova et al., 2018; Jmaeff et al., 2020a, b; Mahato and Sidorova, 2020; Viisanen et al., 2020).

DNSP-11 has been shown to work *in vitro*, however, it was only neuroactive but not neuroprotective on dopaminergic neurons *in vivo* (Fuqua et al., 2014; Stenslik et al., 2015). XIB4035 has been shown to be an allosteric regulator of GDNF increasing GDNF activity (Tokugawa et al., 2003; Hedstrom et al., 2014). Q525 and compound 8 are defined as RET agonists (Jmaeff et al., 2020a, b). The last three molecules are yet to be tested in dopaminergic neurons, so it is not yet clear whether this could translate well into patients.

Two RET agonists, BT13 and BT44, have been shown to stimulate RET both *in vitro* and *in vivo* (Sidorova et al., 2017; Viisanen et al., 2020). It is speculated that BT13 mimics soluble GDNF/GFRα1, rather than GDNF itself, since both proteins may signal in a soluble way. BT13 does not require GFRα co-receptors to elicit its effects and can bind either directly to RET or allosterically modulate the receptor complex to GDNF (Sidorova et al., 2017). The consensus is that RET must homodimerize to become activated. BT13 either induces RET homodimerization by conformational changes or by stabilizing it in its active conformation if the signaling complex is already formed (Bespalov and Saarma, 2007). Striatal delivery of BT13 has been shown to stimulate neuronal signaling and dopamine release similarly to GDNF (Mahato et al., 2020). In contrast to BT13, the third-generation selective RET agonist BT44 has some selectivity to the GFRα1/RET complex when compared to RET alone (Viisanen et al., 2020). This may be beneficial when attempting to ameliorate potential side effects (Viisanen et al., 2020). BT44 is more potent than BT13 but less potent than the GDNF molecule it is mimicking (Sidorova et al., 2017; Mahato and Sidorova, 2020; Viisanen et al., 2020). It was demonstrated that BT44 promotes the survival of primary dopaminergic neurons from wild-type but not RET knockout mice indicating an *in vivo* selectivity of BT44 for RET (Renko et al., 2021). In a 6-hydroxydopamine PD rat model, BT44 protected dopaminergic fibers in the striatum as well as reduced motor imbalance in these animals (Renko et al., 2021). Taking all of these findings together, BT13 and BT44 might have the potential to be developed into further treatments. Unlike GDNF, their production and administration are simpler and may cause fewer side effects, as they seem selective to RET and their signaling might be influenced by the presence of GFRα1 (Jmaeff et al., 2020a, b). This may provide hope to use neurotrophic signaling as a therapeutic approach for PD patients, subverting the requirement for the highly invasive neurosurgery currently required for successful striatal GDNF delivery (Gill et al., 2003; Whone et al., 2019a, b).

Another promising direction to stimulate RET in PD patients might be the use of modified versions of GDNF and neurturin which might diffuse better in the tissue and perhaps even penetrate the blood-brain barrier (Runeberg-Roos et al., 2016; Runeberg-Roos and Penn, 2020). Care must be taken if these proteins are routinely tested preclinically in rhesus monkeys, which have putamen five times smaller than humans, making it difficult to extrapolate their distribution behavior in PD patients (Yin et al., 2009). For example, an N-terminally truncated GDNF variant was created which exhibited a two-fold increase in distribution when administered to rats and non-human primates, whilst compared to primary GDNF molecule retaining its ability to activate the RET receptor complex (Smith et al., 2015; Grondin et al., 2019). These proteins could also be delivered in viral vectors with specific promoters and inducible systems limiting the expression to particular cell types and time points, thus reducing the potential for off-target effects and deleterious overexpression (Chtarto et al., 2016; Kordower, 2016; Axelsen and Woldbye, 2018; Chen et al., 2020; Runeberg-Roos and Penn, 2020). In a phase I clinical trial, treatment with AAV2-GDNF was well tolerated by patients with an enhanced putaminal uptake of $^{18}$F-DOPA suggestive of increased neurotrophic signaling in dopaminergic neurons (Heiss et al., 2019).

## Concluding Remarks

Activating GDNF/RET signaling has yet to show its full beneficial potential in PD patients. Important parameters to consider for further clinical trials are the strata of PD patients, the kind, dose, place, time, and application technique of RET activators. It seems sensible to consider alternative RET stimulators such as small drugs, peptides, and modified GDNF with high RET receptor selectivity, improved tissue distribution properties and the ability to penetrate the blood-brain barrier. Small molecules may additionally be able to treat non-motor symptoms of PD and could be a better option. More preclinical research is needed to carefully evaluate the properties of different RET activators and optimize the parameters for clinical application. Stimulating RET signaling in the gut-brain axis and midbrain dopaminergic system is most likely just one of several approaches which might help to slow down, stop or reverse this devastating neurodegenerative disease. We envision that the majority of PD patients could benefit best from receiving a combination of different treatments and that stimulating RET could be one of them.

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## References

Albert K, Voutilainen MH, Domanskyi A, Airavaara M (2017) AAV vector-mediated gene delivery to substantia nigra dopamine neurons: implications for gene therapy and disease models. Genes (Basel) 8:63.

Axelsen TM, Woldbye DPD (2018) Gene therapy for Parkinson's disease, an update. J Parkinsons Dis 8:195-215.

Backman CM, Shan L, Zhang YJ, Hoffer BJ, Leonard S, Troncoso JC, Vonsatel P, Tomac AC (2006) Gene expression patterns for GDNF and its receptors in the human putamen affected by Parkinson's disease: a real-time PCR study. Mol Cell Endocrinol 252:160-166.

Barker RA, Björklund A, Gash DM, Whone A, Van Laar A, Kordower JH, Bankiewicz K, Kieburtz K, Saarma M, Booms S, Huttunen HJ, Kells AP, Fiandaca MS, Stoessl AJ, Eidelberg D, Federoff H, Voutilainen MH, Dexter DT, Eberling J, Brundin P, et al. (2020) GDNF and Parkinson's disease: where next? a summary from a recent workshop. J Parkinsons Dis 10:875-891.

Barlow A, de Graaff E, Pachnis V (2003) Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 40:905-916.

Bartus RT, Kordower JH, Johnson EM, Jr., Brown L, Kruegel BR, Chu Y, Baumann TL, Lang AE, Olanow CW, Herzog CD (2015) Post-mortem assessment of the short and long-term effects of the trophic factor neurturin in patients with alpha-synucleinopathies. Neurobiol Dis 78:162-171.

Bespalov MM, Saarma M (2007) GDNF family receptor complexes are emerging drug targets. Trends Pharmacol Sci 28:68-74.
Boger HA, Middaugh LD, Zaman V, Hoffer B, Granholm AC (2008) Differential effects of the dopamine neurotoxin MPTP in animals with a partial deletion of the GDNF receptor, GFR alpha1, gene. Brain Res 1241:18-28.
Braak H, Del Tredici K (2017) Neuropathological staging of brain pathology in sporadic Parkinson's disease: separating the wheat from the chaff. J Parkinsons Dis 7(s1):S71-85.
Braak H, de Vos RA, Bohl J, Del Tredici K (2006) Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 396:67-72.
Chen W, Hu Y, Ju D (2020) Gene therapy for neurodegenerative disorders: advances, insights and prospects. Acta Pharm Sin B 10:1347-1359.
Chmielarz P, Er S, Konovalova J, Bandres L, Hlushchuk I, Albert K, Panhelainen A, Luk K, Airavaara M, Domanskyi A (2020) GDNF/RET signaling pathway activation eliminates lewy body pathology in midbrain dopamine neurons. Mov Disord 35:2279-2289.
Chtarto A, Humbert-Claude M, Bockstael O, Das AT, Boutry S, Breger LS, Klaver B, Melas C, Barroso-Chinea P, Gonzalez-Hernandez T, Muller RN, DeWitte O, Levivier M, Lundberg C, Berkhout B, Tenenbaum L (2016) A regulatable AAV vector mediating GDNF biological effects at clinically-approved sub-antimicrobial doxycycline doses. Mol Ther Methods Clin Dev 5:16027.
Conway JA, Ince S, Black S, Kramer ER (2020) GDNF/RET signaling in dopamine neurons in vivo. Cell Tissue Res 382:135-146.
Decressac M, Kadkhodaei B, Mattsson B, Laguna A, Perlmann T, Bjorklund A (2012) alpha-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. Sci Transl Med doi: 10.1126/scitranslmed.3004676.
Decressac M, Ulusoy A, Mattsson B, Georgievska B, Romero-Ramos M, Kirik D, Bjorklund A (2011) GDNF fails to exert neuroprotection in a rat alpha-synuclein model of Parkinson's disease. Brain 134:2302-2311.
Drinkut A, Tillack K, Meka DP, Schulz JB, Kuegler S, Kramer ER (2016) RET is essential to mediate GDNF's neuroprotective and neuroregenerative effect in a Parkinson disease mouse model. Cell Death Dis 7:e2359.
Duffy MF, Collier TJ, Patterson JR, Kemp CJ, Fischer DL, Stoll AC, Sortwell CE (2018) Quality over quantity: advantages of using alpha-synuclein preformed fibril triggered synucleinopathy to model idiopathic Parkinson's disease. Front Neurosci 12:621.
Enterria-Morales D, Lopez-Lopez I, Lopez-Barneo J, d'Anglemont de Tassigny X (2020) Role of glial cell line-derived neurotrophic factor in the maintenance of adult mesencephalic catecholaminergic neurons. Mov Disord 35:565-576.
Fuqua JL, Littrell OM, Lundblad M, Turchan-Cholewo J, Abdelmoti LG, Galperin E, Bradley LH, Cass WA, Gash DM, Gerhardt GA (2014) Dynamic changes in dopamine neuron function after DNSP-11 treatment: effects in vivo and increased ERK 1/2 phosphorylation in vitro. Peptides 54:1-8.
Gash DM, Gerhardt GA, Bradley LH, Wagner R, Slevin JT (2020) GDNF clinical trials for Parkinson's disease: a critical human dimension. Cell Tissue Res 382:65-70.
Georgievska B, Kirik D, Bjorklund A (2004) Overexpression of glial cell line-derived neurotrophic factor using a lentiviral vector induces time- and dose-dependent downregulation of tyrosine hydroxylase in the intact nigrostriatal dopamine system. J Neurosci 24:6437-6445.
Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P (2003) Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat Med 9:589-595.
Greene JG (2014) Causes and consequences of degeneration of the dorsal motor nucleus of the vagus nerve in Parkinson's disease. Antioxid Redox Signal 21:649-667.
Grondin R, Littrell OM, Zhang Z, Ai Y, Huettl P, Pomerleau F, Quintero JE, Andersen AH, Stenslik MJ, Bradley LH, Lemmon J, O'Neill MJ, Gash DM, Gerhardt GA (2019) GDNF revisited: a novel mammalian cell-derived variant form of GDNF increases dopamine turnover and improves brain biodistribution. Neuropharmacology 147:28-36.
Hedstrom KL, Murtie JC, Albers K, Calcutt NA, Corfas G (2014) Treating small fiber neuropathy by topical application of a small molecule modulator of ligand-induced GFRalpha/RET receptor signaling. Proc Natl Acad Sci U S A 111:2325-2330.
Heiss JD, Lungu C, Hammoud DA, Herscovitch P, Ehrlich DJ, Argersinger DP, Sinharay S, Scott G, Wu T, Federoff HJ, Zaghloul KA, Hallett M, Lonser RR, Bankiewicz KS (2019) Trial of magnetic resonance-guided putaminal gene therapy for advanced Parkinson's disease. Mov Disord 34:1073-1078.
Hernando S, Herran E, Figueiro-Silva J, Pedraz JL, Igartua M, Carro E, Hernandez RM (2018) Intranasal administration of TAT-conjugated lipid nanocarriers loading GDNF for Parkinson's disease. Mol Neurobiol 55:145-155.
Hyndman BD, Crupi MJF, Peng S, Bone LN, Rekab AN, Lian EY, Wagner SM, Antonescu CN, Mulligan LM (2017) Differential recruitment of E3 ubiquitin ligase complexes regulates RET isoform internalization. J Cell Sci 130:3282-3296.
Ishiguro Y, Iwashita T, Murakami H, Asai N, Iida K, Goto H, Hayakawa T, Takahashi M (1999) The role of amino acids surrounding tyrosine 1062 in RET in specific binding of the shc phosphotyrosine-binding domain. Endocrinology 140:3992-3998.
Ivanova L, Tammiku-Taul J, Sidorova Y, Saarma M, Karelson M (2018) Small-molecule ligands as potential GDNF family receptor agonists. ACS Omega 3:1022-1030.
Jmaeff S, Sidorova Y, Lippiatt H, Barcelona PF, Nedev H, Saragovi LM, Hancock MA, Saarma M, Saragovi HU (2020a) Small-molecule ligands that bind the RET receptor activate neuroprotective signals independent of but modulated by coreceptor GFRalpha1. Mol Pharmacol 98:1-12.
Jmaeff S, Sidorova Y, Nedev H, Saarma M, Saragovi HU (2020b) Small-molecule agonists of the RET receptor tyrosine kinase activate biased trophic signals that are influenced by the presence of GFRa1 co-receptors. J Biol Chem 295:6532-6542.
Kingsbury AE, Daniel SE, Sangha H, Eisen S, Lees AJ, Foster OJ (2004) Alteration in alpha-synuclein mRNA expression in Parkinson's disease. Mov Disord 19:162-170.
Kirkeby A, Barker RA (2019) Parkinson disease and growth factors-is GDNF good enough? Nat Rev Neurol 15:312-314.
Kopra JJ, Panhelainen A, Af Bjerken S, Porokuokka LL, Varendi K, Olfat S, Montonen H, Piepponen TP, Saarma M, Andressoo JO (2017) Dampened amphetamine-stimulated behavior and altered dopamine transporter function in the absence of brain GDNF. J Neurosci 37:1581-1590.
Kopra J, Vilenius C, Grealish S, Harma MA, Varendi K, Lindholm J, Castren E, Voikar V, Bjorklund A, Piepponen TP, Saarma M, Andressoo JO (2015) GDNF is not required for catecholaminergic neuron survival in vivo. Nat Neurosci 18:319-322.
Kordower JH (2016) AAV2-Neurturin for Parkinson's disease: what lessons have we learned? Methods Mol Biol 1382:485-490.
Kramer ER, Aron L, Ramakers GMJ, Seitz S, Zhuang X, Beyer K, Smidt MP, Klein R (2007) Absence of RET signaling in mice causes progressive and late degeneration of the nigrostriatal system. PLoS Biol 5:616-628.
Kramer ER, Liss B (2015) GDNF-RET signaling in midbrain dopaminergic neurons and its implication for Parkinson disease. FEBS Lett 589:3760-3772.
Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Härma MA, Vilenius C, Lilleväli K, Tekko T, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Strömberg I, et al. (2015) GDNF overexpression from the native locus reveals its role in the nigrostriatal dopaminergic system function. PLoS Genet 11:e1005710.
Lake D, Correa SA, Muller J (2016) Negative feedback regulation of the ERK1/2 MAPK pathway. Cell Mol Life Sci 73:4397-4413.
Lang AE, Gill S, Patel NK, Lozano A, Nutt JG, Penn R, Brooks DJ, Hotton G, Moro E, Heywood P, Brodsky MA, Burchiel K, Kelly P, Dalvi A, Scott B, Stacy M, Turner D, Wooten VG, Elias WJ, Laws ER, et al. (2006) Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann Neurol 59:459-466.
Li K, Zhang K, Xu S, Wang X, Zhou Y, Zhou Y, Gao P, Lin J, Ding G, Guo G (2018) EMP-induced BBB-disruption enhances drug delivery to glioma and increases treatment efficacy in rats. Bioelectromagnetics 39:60-67.
Lo Bianco C, Deglon N, Pralong W, Aebischer P (2004) Lentiviral nigral delivery of GDNF does not prevent neurodegeneration in a genetic rat model of Parkinson's disease. Neurobiol Dis 17:283-289.
Mahato AK, Kopra J, Renko JM, Visnapuu T, Korhonen I, Pulkkinen N, Bespalov MM, Domanskyi A, Ronken E, Piepponen TP, Voutilainen MH, Tuominen RK, Karelson M, Sidorova YA, Saarma M (2020) Glial cell line-derived neurotrophic factor receptor Rearranged during transfection agonist supports dopamine neurons in vitro and enhances dopamine release in vivo. Mov Disord 35:245-255.
Mahato AK, Sidorova YA (2020) Glial cell line-derived neurotrophic factors (GFLs) and small molecules targeting RET receptor for the treatment of pain and Parkinson's disease. Cell Tissue Res 382:147-160.

Manfredsson FP, Polinski NK, Subramanian T, Boulis N, Wakeman DR, Mandel RJ (2020) The future of GDNF in Parkinson's disease. Front Aging Neurosci 12:593572.

Meka DP, Mueller-Rischart AK, Nidadavolu P, Mohammadi B, Motori E, Ponna SK, Aboutalebi H, Bassal M, Annamneedi A, Finckh B, Miesbauer M, Rotermund N, Lohr C, Tatzelt J, Winklhofer KF, Kramer ER (2015) Parkin cooperates with GDNF/RET signaling to prevent dopaminergic neuron degeneration. J Clin Invest 125:1873-1885.

Mijatovic J, Airavaara M, Planken A, Auvinen P, Raasmaja A, Piepponen TP, Costantini F, Ahtee L, Saarma M (2007) Constitutive RET activity in knock-in multiple endocrine neoplasia type B mice induces profound elevation of brain dopamine concentration via enhanced synthesis and increases the number of TH-positive cells in the substantia nigra. J Neurosci 27:4799-4809.

Nosrat CA, Tomac A, Hoffer BJ, Olson L (1997) Cellular and developmental patterns of expression of RET and glial cell line-derived neurotrophic factor receptor alpha mRNAs. Exp Brain Res 115:410-422.

Nutt JG, Burchiel KJ, Comella CL, Jankovic J, Lang AE, Laws ER, Jr., Lozano AM, Penn RD, Simpson RK, Jr., Stacy M, Wooten GF, factor IGSGIiGcl-dn (2003) Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology 60:69-73.

Olgiati S, Thomas A, Quadri M, Breedveld GJ, Graafland J, Eussen H, Douben H, de Klein A, Onofrj M, Bonifati V (2015) Early-onset parkinsonism caused by alpha-synuclein gene triplication: clinical and genetic findings in a novel family. Parkinsonism Relat Disord 21:981-986.

Pedrosa Carrasco AJ, Timmermann L, Pedrosa DJ (2018) Management of constipation in patients with Parkinson's disease. NPJ Parkinsons Dis 4:6.

Polinski NK, Volpicelli-Daley LA, Sortwell CE, Luk KC, Cremades N, Gottler LM, Froula J, Duffy MF, Lee VMY, Martinez TN, Dave KD (2018) Best practices for generating and using alpha-synuclein pre-formed fibrils to model Parkinson's disease in rodents. J Parkinsons Dis 8:303-322.

Quintino L, Avallone M, Brannstrom E, Kavanagh P, Lockowandt M, Garcia Jareno P, Breger LS, Lundberg C (2019) GDNF-mediated rescue of the nigrostriatal system depends on the degree of degeneration. Gene Ther 26:57-64.

Renko JM, Mahato AK, Visnapuu T, Valkonen K, Karelson M, Voutilainen MH, Saarma M, Tuominen RK, Sidorova YA (2021) Neuroprotective potential of a small molecule RET Agonist in cultured dopamine neurons and hemiparkinsonian rats. J Parkinsons Dis doi: 10.3233/JPD-202400.

Runeberg-Roos P, Penn RD (2020) Improving therapeutic potential of GDNF family ligands. Cell Tissue Res 382:173-183.

Runeberg-Roos P, Piccinini E, Penttinen AM, Matlik K, Heikkinen H, Kuure S, Bespalov MM, Peranen J, Garea-Rodriguez E, Fuchs E, Airavaara M, Kalkkinen N, Penn R, Saarma M (2016) Developing therapeutically more efficient Neurturin variants for treatment of Parkinson's disease. Neurobiol Dis 96:335-345.

Schaeffer E, Kluge A, Bottner M, Zunke F, Cossais F, Berg D, Arnold P (2020) Alpha synuclein connects the gut-brain axis in Parkinson's disease patients - a view on clinical aspects, cellular pathology and analytical methodology. Front Cell Dev Biol 8:573696.

Schlee S, Carmillo P, Whitty A (2006) Quantitative analysis of the activation mechanism of the multicomponent growth-factor receptor RET. Nat Chem Biol 2:636-644.

Siddiqui MF, Rast S, Lynn MJ, Auchus AP, Pfeiffer RF (2002) Autonomic dysfunction in Parkinson's disease: a comprehensive symptom survey. Parkinsonism Relat Disord 8:277-284.

Sidorova YA, Bespalov MM, Wong AW, Kambur O, Jokinen V, Lilius TO, Suleymanova I, Karelson G, Rauhala PV, Karelson M, Osborne PB, Keast JR, Kalso EA, Saarma M (2017) A novel small molecule gdnf receptor RET agonist, BT13, promotes neurite growth from sensory neurons in vitro and attenuates experimental neuropathy in the rat. Front Pharmacol 8:365.

Sidorova YA, Saarma M (2020) Can growth factors cure Parkinson's disease? Trends Pharmacol Sci 41:909-922.

Slevin JT, Gash DM, Smith CD, Gerhardt GA, Kryscio R, Chebrolu H, Walton A, Wagner R, Young AB (2007) Unilateral intraputamenal glial cell line-derived neurotrophic factor in patients with Parkinson disease: response to 1 year of treatment and 1 year of withdrawal. J Neurosurg 106:614-620.

Slevin JT, Gerhardt GA, Smith CD, Gash DM, Kryscio R, Young B (2005) Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor. J Neurosurg 102:216-222.

Smith RC, O'Bryan LM, Mitchell PJ, Leung D, Ghanem M, Wilson JM, Hanson JC, Sossick S, Cooper J, Huang L, Merchant KM, Lu J, O'Neill MJ (2015) Increased brain bio-distribution and chemical stability and decreased immunogenicity of an engineered variant of GDNF. Exp Neurol 267:165-176.

Stenslik MJ, Potts LF, Sonne JW, Cass WA, Turchan-Cholewo J, Pomerleau F, Huettl P, Ai Y, Gash DM, Gerhardt GA, Bradley LH (2015) Methodology and effects of repeated intranasal delivery of DNSP-11 in a rat model of Parkinson's disease. J Neurosci Methods 251:120-129.

Su X, Fischer DL, Li X, Bankiewicz K, Sortwell CE, Federoff HJ (2017) Alpha-Synuclein mRNA is not increased in sporadic PD and alpha-synuclein accumulation does not block GDNF signaling in Parkinson's disease and disease models. Mol Ther 25:2231-2235.

Taraviras S, Marcos-Gutierrez CV, Durbec P, Jani H, Grigoriou M, Sukumaran M, Wang LC, Hynes M, Raisman G, Pachnis V (1999) Signalling by the RET receptor tyrosine kinase and its role in the development of the mammalian enteric nervous system. Development 126:2785-2797.

Tofaris GK, Kim HT, Hourez R, Jung JW, Kim KP, Goldberg AL (2011) Ubiquitin ligase Nedd4 promotes alpha-synuclein degradation by the endosomal-lysosomal pathway. Proc Natl Acad Sci U S A 108:17004-17009.

Tokugawa K, Yamamoto K, Nishiguchi M, Sekine T, Sakai M, Ueki T, Chaki S, Okuyama S (2003) XIB4035, a novel nonpeptidyl small molecule agonist for GFRalpha-1. Neurochem Int 42:81-86.

Turconi G, Kopra J, Voikar V, Kulesskaya N, Vilenius C, Piepponen TP, Andressoo JO (2020) Chronic 2-fold elevation of endogenous GDNF levels is safe and enhances motor and dopaminergic function in aged mice. Mol Ther Methods Clin Dev 17:831-842.

Uesaka T, Jain S, Yonemura S, Uchiyama Y, Milbrandt J, Enomoto H (2007) Conditional ablation of GFRalpha1 in postmigratory enteric neurons triggers unconventional neuronal death in the colon and causes a Hirschsprung's disease phenotype. Development 134:2171-2181.

Viisanen H, Nuotio U, Kambur O, Mahato AK, Jokinen V, Lilius T, Li W, Santos HA, Karelson M, Rauhala P, Kalso E, Sidorova YA (2020) Novel RET agonist for the treatment of experimental neuropathies. Mol Pain 16:1744806920950866.

Volinsky N, Kholodenko BN (2013) Complexity of receptor tyrosine kinase signal processing. Cold Spring Harb Perspect Biol 5:a009043.

Wallen AA, Castro DS, Zetterstrom RH, Karlen M, Olson L, Ericson J, Perlmann T (2001) Orphan nuclear receptor Nurr1 is essential for RET expression in midbrain dopamine neurons and in the brain stem. Mol Cell Neurosci 18:649-663.

Whone AL, Boca M, Luz M, Woolley M, Mooney L, Dharia S, Broadfoot J, Cronin D, Schroers C, Barua NU, Longpre L, Barclay CL, Boiko C, Johnson GA, Fibiger HC, Harrison R, Lewis O, Pritchard G, Howell M, Irving C, et al. (2019a) Extended treatment with glial cell line-derived neurotrophic factor in Parkinson's disease. J Parkinsons Dis 9:301-313.

Whone A, Luz M, Boca M, Woolley M, Mooney L, Dharia S, Broadfoot J, Cronin D, Schroers C, Barua NU, Longpre L, Barclay CL, Boiko C, Johnson GA, Fibiger HC, Harrison R, Lewis O, Pritchard G, Howell M, Irving C, et al. (2019b) Randomized trial of intermittent intraputamenal glial cell line-derived neurotrophic factor in Parkinson's disease. Brain 142:512-525.

Yin D, Valles FE, Fiandaca MS, Forsayeth J, Larson P, Starr P, Bankiewicz KS (2009) Striatal volume differences between non-human and human primates. J Neurosci Methods 176:200-205.

Yoshii SR, Kishi C, Ishihara N, Mizushima N (2011) Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. J Biol Chem 286:19630-19640.

Zaman V, Boger HA, Granholm AC, Rohrer B, Moore A, Buhusi M, Gerhardt GA, Hoffer BJ, Middaugh LD (2008) The nigrostriatal dopamine system of aging GFRalpha-1 heterozygous mice: neurochemistry, morphology and behavior. Eur J Neurosci 28:1557-1568.

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