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Bioinformatics and Immunohistochemical Analyses Support Preserved Expression of Glial Cell Line–Derived Neurotrophic Factor Receptor RET in Parkinson's

Intracerebral administration of glial cell line–derived neurotrophic factor (GDNF) as a disease-modifying therapy has been trialed in Parkinson's disease (PD)[1,2]; however, the potent effects seen in animal models have not been reproduced clinically. One reason proposed for this lack of efficacy in humans is downregulation of the GDNF receptor RET by α-synuclein (αSyn) in the PD substantia nigra (SN),[3] which is supported by some,[4,5] but not all,[6] preclinical studies. The recent description of off-state dyskinesia correlating with GDNF administration in a clinical trial participant[7] may be evidence of functional pharmacological effects of GDNF, even in advanced PD. In this letter, we consider these findings in light of gene expression analysis using open-source transcriptome data and immunohistochemistry of RET expression in the SN of patients with PD.

We used the R package *limma* to identify differentially expressed genes (DEGs) with $\log_2$FC (fold change) of ±0.4 and false discovery rate (FDR) adjusted $P \leq 0.05$, between PD and control SN, using a combined dataset comprising data from five microarray studies: GSE8397, GSE7621, GSE49036, GSE2029, and GSE20186 (see Supporting Information Methods in Data S1). Of the 142 samples, 79 were PD cases and 63 were controls. The most significant DEG was the dopamine transporter *SLC18A2* (*VMAT2*), with a logFC value of −2.34 in PD SN samples compared with controls (FDR = 9.57E−10). Notably, throughout the DEG list there was significant downregulation of dopaminergic markers in the PD group, including downregulation of *RET* (logFC = −1.62, FDR = 1.08E−07) (Fig. 1A,B).

To investigate whether this downregulation of *RET* expression in PD was due to, or independent of, the loss of dopamine neurons, we examined the coexpression of *RET* and seven dopaminergic markers: *SLC18A2*, *TH*, *DDC*, *SLC6A3*, *KCNJ6*, *ALDH1A1*, and *NR4A2*. For the majority of comparisons, there was significant moderate to strong correlation between *RET* and a given marker in controls, and this correlation was maintained in PD (Fig. 1C,D). This suggests that the downregulation of *RET* observed in the PD SN is likely due to dopaminergic neuronal loss, rather than downregulation of *RET* transcript expression. To extend these findings, we performed differential expression analysis using data generated from laser-captured microdissection (LCM) of individual SN neurons in postmortem brains of 10 PD and 8 control cases (GSE20141). In agreement with the coexpression data, *RET* was not significantly downregulated in the LCM dataset (Fig. 1E).

Our immunohistochemical analysis found that RET expression was reduced in SN neurons in PD compared with controls, but that RET continued to be expressed even in late-stage PD (Fig. 1F,G). These data are in agreement with a recent study[3] and suggest that the receptor needed for GDNF to be effective is intact in the PD brain, even at late-stage disease. The report by Lloyd et al[7] suggested that intracerebral GDNF is capable of inducing sprouting, even in cells carrying αSyn. To test this, we transduced primary cultures of embryonic day 14 rat ventral mesencephalon with AAV-GFP or AAV-αSyn and found that, although αSyn significantly reduced neurite growth after 10 days, GDNF treatment led to a significant increase in neurite growth (Fig. 1H). Our study shows sustained, albeit reduced, expression of RET in the PD SN and is consistent with the report Lloyd et al,[7] whose results suggest that GDNF may induce sprouting of dopaminergic terminals throughout the putamen. Our finding that RET expression is retained in the PD SN, albeit at a lower level than in controls, provides further support for the future of GDNF therapy. Nevertheless, early administration of GDNF, when extensive loss of RET-expressing dopaminergic neurons has not yet occurred, may lead to better outcomes in patients with PD.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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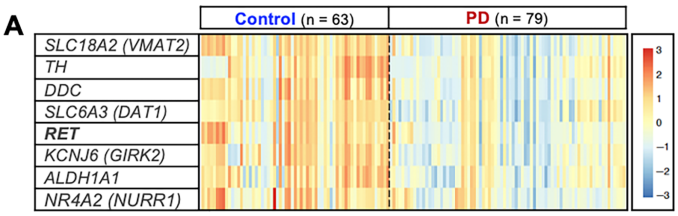
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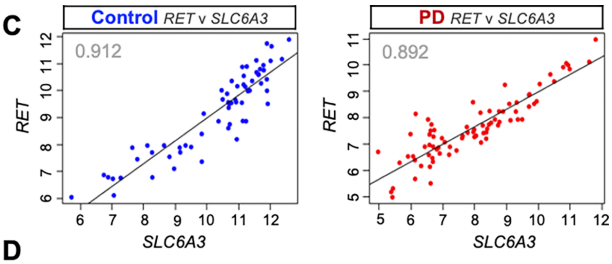
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B

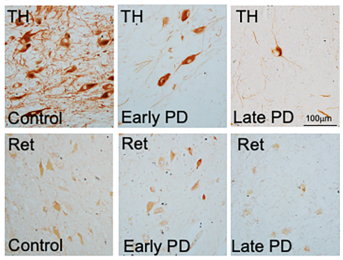
Symbol	Gene name	Log FC	*t*	*FDR*
SLC18A2	Solute Carrier Family 18 Member A2	−2.34	−8.31	9.57E−10
TH	Tyrosine Hydroxylase	−1.90	−6.05	1,88E−06
DDC	Dopa Decarboxylase	−1.89	−7.47	1.40E−08
SLC6A3	Solute Carrier Family 6 Member 3	−1.76	−7.63	1.10E−08
RET	**Proto-oncogene tyrosine-protein kinase receptor Ret**	**−1.62**	**−6.94**	**1.08E−07**
KCNJ6	K+ Inwardly Rectifying Channel Subfamily J member ,6	−1.55	−7.16	4.78E−08
ALDH1A1	Aldehyde dehydrogenase 1 A1	−1.47	−6.04	1.93E−06
NR4A2	Nuclear receptor-related factor 1	−1.05	−6.15	1.35E−06

D

Gene co-expression in SN	Control		PD	
	Pearson's Correlation	*P* value	Pearson's Correlation	*P* value
RET v *SLC18A2*	0.912	2.63E−25	0.892	2.51E−28
RET v *TH*	0.379	2.20E−03	0.650	9.18E−11
RET v *DDC*	0.803	2.39E−15	0.866	7.19E−25
RET v *SLC6A3*	0.682	7.63E−10	0.687	2.70E−12
RET v *KCNJ6*	0.783	3.23E−14	0.796	1.76E−18
RET v *ALDH1A1*	0.749	1.65E−12	0.674	9.85E−12
RET v *NR4A2*	0.871	1.78E−20	0.717	1.05E−13

E

Individual DA neurons (GSE20141)			
Symbol	**Log FC**	***t***	***FDR***
SLC18A2	−1.09	−0.99	0.631
TH	−0.33	−0.56	0.803
DDC	−0.97	−1.26	0.540
SLC6A3	0.64	0.42	0.706
RET	**−0.84**	**−1.39**	**0.504**
KCNJ6	−0.72	−0.80	0.709
ALDH1A1	−1.08	−1.18	0.564
NR4A2	−1.05	−1.98	0.379



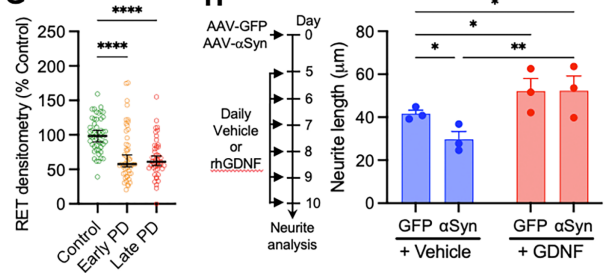


FIG. 1. Bioinformatics and immunohistochemical analysis show that RET expression is decreased but still present in dopaminergic neurons in the substantia nigra (SN) in Parkinson's disease (PD). (**A**) Heatmap showing gene expression in SN of control and PD samples, ranked by logFC. Individual SN tissue samples are represented by each of the 142 columns of expression values within the heatmap. (**B**) List of the top differentially expressed genes, ordered by logFC, in the SN of PD compared with controls. A minus symbol denotes reduced in PD. (**C**) Scatterplot showing linear regression analysis of RET and SLC6A3 (DAT) in control and PD SN samples. (**D**) Table showing Pearson correlation coefficients and *P* values for the comparison between *RET* and multiple dopaminergic markers in the SN of PD and controls, from merged datasets. (**E**) Table showing expression of the same genes in (**B**), analyzed in expression data generated from laser-captured microdissection (LCM) of individual SN neurons in postmortem brains of 10 PD and 8 control cases (GSE20141). (**F**) Representative photomicrographs of TH and RET staining in formalin-fixed paraffin-embedded SN sections from control, early-stage, and late-stage PD cases. (**G**) Graph showing quantification of RET immunoreactivity in the SN of control, early-stage, and late-stage PD cases. Data are presented as median ± 95% confidence interval from n = 50 neurons per group (10 RET-immunoreactive neurons per SN, 5 SN samples per group). Individual data points represent individual neurons. One-way ANOVA with Tukey's post hoc test (****$P < 0.0001$). (**H**) Schema and graph of length of the longest neurite in primary cultures of E14 rat VM, transduced with AAV-GFP and AAV–α-synuclein and treated with 100 ng/mL glial cell line–derived neurotrophic factor (GDNF) on day 5 and daily thereafter for an additional 5 DIV (experimental end point day 10). Data are presented as mean ± SEM from n = 3 independent experiments. Two-way ANOVA with Tukey's post hoc test (*$P < 0.05$, **$P < 0.01$). DAT, dopamine transporter; DIV, days in vitro; E14, embryonic day 14; TH, tyrosine hydroxylase; VM, ventral mesencephalon. [Color figure can be viewed at wileyonlinelibrary.com]

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Supporting Data

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