Expanded Study of NURR1 gene expression in patients with Parkinson's disease

Weidong Le[1]; Tianhong Pan[1]; Wenjie Xie; Maosheng Huang ; Pingyi Xu; Wen Zhu; Xiong Zhang; Hao Deng; Joseph Jankovic*
Parkinson Disease Research Laboratory, Parkinson's Disease Center and Movement Disorder Clinic, Department of Neurology, Baylor College of Medicine, Houston, 77030, Texas, USA

ABSTRACT

Objective: NURR1 is a transcription factor essential for the development, survival of midbrain dopaminergic (DAergic) neurons and *NURR1* is a potential susceptibility gene for Parkinson's disease (PD). To explore the relevance of peripheral *NURR1* gene expression in PD, we conducted a pilot study and found a significant reduction in *NURR1* mRNA levels in peripheral blood lymphocytes (PBL) of 113 PD patients vs 42 healthy control (HC). To validate this finding we designed another study in a double-blind manner in a larger population of PD patients, HC, and various NDC. The aims of this study were to determine **(1)** whether *NURR1* gene expression in PBL is specifically reduced in PD as compared with HC and NDC; **(2)** whether the *NURR1* expression can be used to help identify PD, and **(3)** whether age, gender, anti-PD medications, or disease severity affects the expression of *NURR1*. **Methods:** We measured *NURR1* expression in PBL in 278 patients with PD, 166 healthy controls (HC), and 256 neurological disease controls (NDC) by quantitative real-time PCR in a blind fashion. **Results:** *NURR1* gene expression was significantly decreased in patients with PD (particularly those with family history of PD) as compared with HC ($p < 0.01$) and also as compared with NDC ($p < 0.05$). There was no significant difference in *NURR1* gene expression among PD patients with or without anti-PD medications. When adjusted for gender, age, and ethnicity, lower levels of *NURR1* gene expression were associated with significantly increased risk for PD in women, in patients 60 years old or older, and in patients of Caucasian origin. **Conclusions:** We have demonstrated a significant reduction in PBL *NURR1* gene expression in PD patients, indicating possible systemic involvement in PD, and the finding may help identify individuals with PD and other disorders associated with impaired central DAergic system.

BACKGROUND

PD is a common neurodegenerative disease in adults. Because of the inherent difficulty in studying PD pathogenic mechanisms in the central nervous system (CNS), research interests have, therefore, focused on other means of early diagnosis, including cerebrospinal fluid proteomics, serum or plasma metabolomics, and gene expression profile in PBL in an attempt to identify potential peripheral biomarkers of the disease. *NURR1*, a member of the steroid/thyroid nuclear superfamily is essential for the development, survival, and functional maintenance of midbrain DAergic neurons. It is highly expressed in midbrain DAergic neurons as well as other tissues, including PBL. We previously showed that *NURR1*-null mice have selective agenesis of DAergic neurons in the SN and ventral tegmental area. We have also demonstrated that aged heterozygous *NURR1*-null mice (*NURR1*-/-) have fewer DAergic neurons in the SN and reduced expression of *NURR1* increased the vulnerability of mesencephalic DAergic neurons to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced injury. Furthermore, our genetic studies and other reports in patients with PD have indicated that *NURR1* might be a potential susceptibility gene for PD. Our initial open labeled study showed PD patients have significant lower levels of NURR1 mRNA in the PBL. In this study we attempt to validate this finding by double blind fashion in a larger population of PD and control subjects.

METHODS

Subjects

We collected a total of 700 PBL samples: 278 from patients with PD (sporadic PD 178; familial PD 100), 166 from HC and 256 from various non-PD NDC which consist of 53 non-movement neurological disorders, and 203 movement disorder controls. Blood samples were collected at the Parkinson's Disease Center and Movement Disorders Clinic (PDCMDC), Baylor College of Medicine (BCM).

Materials and Procedures

Human peripheral blood was drawn from cubital vein into a heparinized plastic syringe and PBL separation using Ficoll/Paque method. Total RNA was extracted from PBL by spin or vacuum total RNA isolation system. One microgram of total RNA from PBL was reverse transcribed into first-strand cDNA by using iScriptTm cDNA synthesis kit.

Real-time PCR assay of NURR1 gene expression

The fluorescent real-time PCR reaction was carried out in the Bio-Rad iCycler System (Bio-Rad) with a final volume of 25 µl for each reaction containing with the specific primers targeting human *NURR1* and *GAPDH* that was used as internal control. PCR products were detected by the fluorescent probe 5'6-FAM for *NURR1* and 5'Texas red for *GAPDH*. The value of threshold cycle (Ct) was generated at every cycle during a run. Fluorescent reading from real-time PCR reaction was quantitatively analyzed by determining the difference of Ct (delta Ct) between Ct of *NURR1* and *GAPDH*.

Statistical analysis

The X2 test was used to test for differences between the PD patients and the controls in the distributions of gender and ethnicity. A student's t test or a Mann-Whitney test was used for differences between the two groups in the distribution of age and gene expression. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated as estimates of relative risk. One- or two-way ANOVA was performed to evaluate the differences of the relative *NURR1* gene expression.

RESULTS

NURR1 gene expression on PBL of all study groups

The mean level of *NURR1* gene expression in PBL in patients with PD was 52% lower than healthy controls ($p < 0.01$), and 25% lower than all NDC ($p < 0.05$; Figure 1; Table 1). The decreased expression in *NURR1* gene in PD was more robust in patients with familial history of PD and the difference between sPD and fPD was statistically significant ($p < 0.05$; Table 2). There was also a significant difference of *NURR1* expression when HC and NDC were compared ($p < 0.05$; Figure 1; Table 1) and when PD and other movement disorders were compared ($p < 0.05$; Table 1).

PD risk estimate after adjusting age, gender and ethnicity

Since the demographic distribution of gender, age and ethnicity was significantly different among all study groups, we determined the odds ratio (OR) values to estimate the relative risk of PD after adjusting for age, gender and ethnicity. Using HC as reference, we found that the OR was significantly increased in the overall PD population ($p < 0.001$). The estimated relative risk for patients with PD was significantly increased in both male ($p < 0.05$) and female patients ($p < 0.01$), but was greater in females (Table 1 and Table 2). The risk effect of the level of *NURR1* gene expression appeared to be greater in older subjects ($p < 0.01$, age $\geq$ 60 years, and $p < 0.05$ age <60 years), and in subjects of Caucasian origin ($p < 0.001$). No difference in risk of was found in non-movement disorder controls or other movement disorder controls when adjusted for age and gender (Table 2).

The influence of medication

Among 278 PD patients, 65 were of recent-onset and not yet treated with anti-PD medications ("*de novo*" PD), the remaining 213 patients were treated with anti-PD medications, including 58 treated with DA receptor agonists, usually pramipexole or ropinirole, 66 were treated with l-dopa, and other 89 patients were treated with the combination of DA agonists and l-dopa. There was no significant influence of medication on *NURR1* expression.

Correlation of disease duration and severity with NURR1 expression in PD

We performed a correlation analysis between disease duration (years after onset of disease symptoms) and severity (total UPDRS score) in 278 patients with PD. There was no significant correlation between the duration of PD and the level of *NURR1* gene expression (r=-0.03, $p > 0.05$), and there was no correlation between the *NURR1* gene expression and UPDRS scores (r=-0.02, $p > 0.05$).

FIGURES AND TABLES

Figure 1

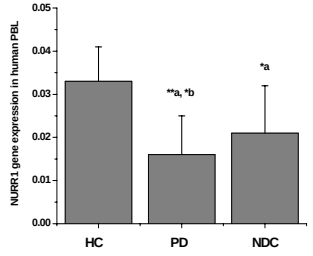

Table 1

Group	Number	NURR1 mRNA mean ± SEM	*P* value	*P* value	*P* value
Healthy control	166	0.033 ± 0.008	Reference		
PD	278	0.016 ± 0.009	<0.01	Reference	
sPD	178	0.018 ± 0.008	<0.05	NA	Reference
fPD	100	0.013 ± 0.010	<0.01	NA	<0.05
NDC	256	0.021±0.011	<0.05	<0.05	NA
Non-MDC	53	0.019 ± 0.014	NS	NS	NA
MDC	203	0.022 ± 0.012	<0.05	<0.05	NA
ET	120	0.027 ± 0.018	NS	<0.05	NA
RLS	41	0.009 ± 0.007	<0.05	NS	NA
Parkinsonism	21	0.022 ± 0.016	NS	NS	NA
Dystonia	21	0.013 ± 0.010	NS	NS	NA

Table 2

PD-OR			Non-MDC-OR			MDC-OR		
Risk estimate in HC and PD groups			Risk estimate in HC and NDC groups			Risk estimate in HC and NDC groups		
	OR (95% CI)			OR (95% CI)			OR (95% CI)	
Overall:			Overall:			Overall:		
	1.66 (1.02-2.10)	<0.001			NS			NS
Gender			Gender			Gender		
Male	1.44 (0.99-1.89)	<0.05	Male		NS	Male		NS
Female	1.78 (1.01-2.16)	<0.01	Female		NS	Female		NS
Age			Age			Age		
<60y	1.69 (1.00-2.21)	<0.05	<60y		NS	<60y		NS
>=60y	1.85 (1.00-2.29)	<0.01	>=60y		NS	>=60y		NS
Race			Race			Race		
Caucasian	1.56 (1.02-2.10)	<0.001	Caucasian		NS	Caucasian		NS
Other		NS	Other		NS	Other		NS

LEGENDS

Fig. 1. Figure 1. *NURR1* gene expression on PBL in different study groups. Fluorescent reading from real-time PCR reaction was quantitatively analyzed by determining the difference of Ct between *NURR1* and *GAPDH*. The level of *NURR1* gene expression was markedly low in patients with PD and moderately low in various NDC vs HC. Data presented as mean ± SEM from a total of 700 subjects in duplication assay measured in blind fashion. * $p < 0.05$ ** $p < 0.01$ vs. HC (a), vs. NDC (b).

Table 1. Data are the means ± SEM from 700 subjects in duplication assay and mRNA levels were determined in blind fashion. Fluorescent reading from real-time PCR reaction was quantitatively analyzed.. sPD=sporadic PD; fPD=familial PD; NDC = neurological disease control; Non-MDC = non-movement disease control; MDC = movement disease control; ET = essential tremor; RLS=rest leg syndrome; NA= Not analyzed; NS = not significant. *Wilcoxon Rank-Sum (Mann-Whiney Test).

Table 2. OR = odds ratio; NS = not significant. The relative risks for patients with PD, NDC, non-movement disease controls (Non-MDC), and movement disease controls (MDC) were estimated by OR value in overall sample after adjusted by age, gender and ethnicity.

CONCLUSIONS

- Although *NURR1* expression is significantly decreased in our PD patients vs HC and disease controls this abnormality may not be specific for PD as it may be also found, although to a lesser degree, in other movement disorders.
- The changes in *NURR1* expression do not seem to correlate with disease progression.
- patients with familial history seem to correlate with greater reduction in PBL *NURR1* expression. Levels of *NURR1* gene expression seem to be lower in women, in patients of 60 years old or older, and in subjects of Caucasian origin.
- Anti-PD medications have limited or no impact on *NURR1* expression.

REFERENCES

- Pan T, et al. Decreased Nurr1 mRNA in peripheral blood lymphocytes in Parkinson's disease. Neurol. 2004; 62 (Suppl 5):A108
- Jankovic J, et al. The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. Prog Neurobiol 2005;77:128-38.
- Chu Y, et al. Nurr1 in Parkinson's disease and related disorders. J Comp Neurol. 2006; 494:495-514.
- Tokuda, et al. Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. Biochem Biophys Res Commun. 2006;349(1):162-6.
- Annanmaki T, et al. Low plasma uric acid level in Parkinson's disease. Mov Disord 2007; 22:1133-1137
- Scherzer CR, et al. Molecular markers of early Parkinson's disease based on gene expression in blood. Proc Natl Acad Sci U S A. 2007;104::955-60
- Bogdanov M, et al. Metabolomic profiling to develop blood biomarkers for Parkinson's disease. Brain 2008; 131:389-396
- Jacobsen KX, et al. A Nurr1 point mutant, implicated in Parkinson's disease, uncouples ERK1/2-dependent regulation of tyrosine hydroxylase transcription. Neurobiol Dis. 2008; 29:117-122.

Study supported by research grants from M J Fox Foundation, the American Parkinson Disease Association, NIH (NINSD-043567) and Diana Helis Henry Medical Research Foundation.