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 (I) whether NURR1 gene expression in PBL is specifically reduced in PDL as compared with HC and NDC; (II) whether the NURR1 expression can be used to help identify PD, and (III) 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 HC, and 256 various non-PD NDC which consist of 53 non-movement neurological disorders.

Materials and Procedures

Human peripheral blood was drawn from cubital vein into a heparinized plastic syringe and PBL separation was performed 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 iScript™ cDNA synthesis kit.

Results

Real-time PCR assay of NURR1 gene expression

The fluorescence 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’-FAM for NURR1 and 5’Texas red for GAPDH. The value of threshold cycle (Ct) was generated at the cycle number of a real-time PCR reaction. Fluorescent reading from real-time PCR reaction was quantitatively analyzed by determining the difference of Ct (delta Ct) between 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 (CI) were calculated as estimates of relative risk. One- or two-way ANOVA was performed to evaluate the differences of the relative NURR1 gene expression. 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).

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