Brain Research 1683 (2018) 12–16

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

CSF lamp2 concentrations are decreased in female Parkinson's disease patients with LRRK2 mutations

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ARTICLE INFO

Article history:
Received 13 November 2017
Received in revised form 10 January 2018
Accepted 15 January 2018

Keywords:
Autophagy
Cerebrospinal fluid
Lamp2
LRRK2
Oxidative stress
Parkinson's disease

ABSTRACT

Lysosome-associated membrane glycoprotein 2 (lamp2) plays critical roles in chaperone-mediated autophagy (CMA) and macroautophagy. Its isoform lamp2a is decreased in Parkinson's disease (PD) substantia nigra. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most known common cause of late-onset PD; although LRRK2 is thought to regulate macroautophagy, the influence of LRRK2 mutations on lamp2 concentrations in the CNS is unknown. To examine this issue we compared lamp2 levels in cerebrospinal fluid (CSF) between sporadic PD (sPD) patients (n = 31), LRRK2 PD patients (n = 20), and healthy control subjects with or without LRRK2 mutations (LRRK2 CTL = 30, CTL = 27). We also examined lamp2's correlations with age, oxidative stress, PD progression, and PD duration. Median lamp2 concentrations (pg/mL) were LRRK2 PD = 127, sPD = 333, CTL = 436, and LRRK2 CTL = 412. Log-transformed lamp2 concentrations, adjusting for gender effects (and excluding male LRRK2 PD patients because of low number), were lower in female LRRK2 PD patients than in LRRK2 CTL ($p = 0.002$) and CTL ($p = 0.005$) subjects ($p = 0.06$ for lamp2 comparison between female LRRK2 PD patients and sPD patients). Lamp2 did not appear to be associated with age, PD progression, or PD duration; however, three of four Spearman rho values for correlations between lamp2 and oxidative stress markers in PD subjects were ≥ 0.30. These findings suggest that CSF lamp2 concentrations may be decreased in female LRRK2 PD patients compared to healthy individuals with or without LRRK2 mutations.

1. Introduction

Lysosome-associated membrane glycoprotein 2 (lamp2) is a ~45 kDa protein which contributes to normal lysosomal functioning (Eskelinen, 2006), including maintenance of lysosomal stability (Saftig and Klumperman, 2009) and protection of the inner surface of lysosomal membranes from hydrolytic enzymes (Fukuda, 1991). Lamp2's three isoforms, lamp2a, lamp2b and lamp2c, are generated by alternative splicing of the lamp2 gene (Gough et al., 1995). All three isoforms are involved in autophagy (Cuervo and Dice, 2000; Nishino et al., 2000; Cuervo et al., 2004; Kiffin et al., 2007; Fujiwara et al., 2015; Sala et al., 2016). Lamp2 is involved in other processes including antigen presentation (Zhou et al., 2005), vasculitis (Kain et al., 2008), endosomal/lysosomal cholesterol trafficking (Eskelinen et al., 2004), and regulation of T lymphocyte responses (Valdor et al., 2014). Lamp2 was shown to mediate oxidative stress-dependent cell death in Zn^{2+}-treated lung epithelial cells (Qin et al., 2017), although whether it plays a role in oxidative stress in the CNS, where it is ubiquitously expressed (Rothaug et al., 2015), is unknown. Lamp2 concentrations in cerebrospinal fluid (CSF) have been reported to be reduced in patients with Parkinson's disease (PD) (Boman et al., 2016) and increased in patients with Alzheimer's disease (Armstrong et al., 2014; Sjödin et al., 2016).

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most common known cause of late-onset PD (Tong et al.,2012). Of relevance to lamp2 is that LRRK2 is thought to regulate macroautophagy (Gómez-Suaga et al., 2012a,b; Yang et al., 2014). However, the influence of PD-associated LRRK2 mutations on lamp2 levels in the CNS are unknown. Our primary objective in this study was to compare CSF lamp2 concentrations between patients with sporadic PD (sPD), PD patients with LRRK2 mutations (LRRK2 PD), and similar-aged healthy control subjects with or without PD-associated LRRK2 mutations (LRRK2 CTL, CTL). Our secondary objective was to examine lamp2's associations with age and the oxidative stress markers 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostane (8-ISO) in these groups, and its associations with

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https://doi.org/10.1016/j.brainres.2018.01.016
0006-8993/

Unified Parkinson's Disease Rating Scale (UPDRS) Total and Part 3 scores, commonly used to monitor PD's clinical progression (Fahn et al., 1987), and duration of clinical disease in our sPD and LRRK2 PD patients.

2. Results

2.1. Study subjects

Summary statistics of demographic and clinical characteristics for the 108 study subjects, distributed by diagnostic group, are shown in Table 1. There were 67 women (62%) and 41 men (38%) in this study, with a mean age of 61.5 years (SD 9.4, range 44–85). The diagnostic groups were well balanced for age. Gender distribution was similar between the sPD, LRRK2 CTL, and CTL groups (48%, 40%, and 37% men) but the LRRK2 PD group had a smaller proportion of men (4/20 = 20%).

2.2. Separate effects of diagnostic group and gender on CSF lamp2 concentrations

Median lamp2 concentrations, expressed as pg/mL, for the diagnostic groups were sPD = 333 (range: 52.4–4542), LRRK2 PD = 127 (52.4–2047), CTL = 436 (52.4–10,561), and LRRK2 CTL = 412 (52.4–5695). Female subjects tended to have lower lamp2 concentrations than male subjects; median lamp2 concentrations were 247 for females and 778 pg/mL for males. Boxplots of summary statistics for lamp2 concentrations in the diagnostic groups indicated that the data were not normally distributed (Fig. 1). After natural logarithmic transformation of lamp2 concentrations, the data were reasonably normally distributed with similar variances between groups. Summary statistics for log-transformed lamp2 concentrations are shown in Fig. 2 for the diagnostic groups, and in Fig. 3 for combinations of gender with diagnostic group, excluding the four male LRRK2 PD patients.

2.3. Combined effects of diagnostic group and gender on CSF lamp2 concentrations

Because lamp2 concentrations appeared to vary with both diagnostic group and gender, inference was based on a two-way ANOVA which simultaneously modeled the effects of diagnostic group and gender on log-transformed lamp2 concentrations. LRRK2 PD males were excluded from the analysis because of their low number. After excluding this group, there was no evidence of an interaction between gender and diagnostic group ($p = 0.63$). The adjusted (least squares) mean values for log-transformed lamp2 concentrations in the diagnostic groups were sPD = 5.87, LRRK2 PD = 4.94, CTL = 6.19, LRRK2 CTL = 6.31; mean values were 5.57 for female subjects and 6.08 for male subjects. Significant effects on

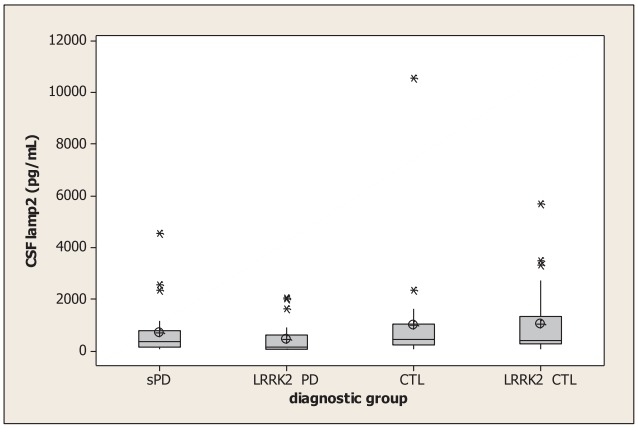

Fig. 1. Distribution of CSF lamp2 concentrations in diagnostic groups. Means (circle), medians (line through center of box), upper and lower quartiles (upper and lower borders of box, respectively), most extreme non-outlier values (lines extending from box), and outliers (asterisks) are shown for lamp2 concentrations (non-log-transformed) in CSF specimens from sPD, LRRK2 PD, CTL, and LRRK2 CTL subjects. The distribution for each group was skewed right, with outliers. (sPD, sporadic Parkinson's disease; LRRK2 PD, Parkinson's disease subjects carrying LRRK2 gene mutations; CTL, healthy control subjects lacking detectable PD-associated gene mutations; LRRK2 CTL, healthy control subjects carrying PD-associated LRRK2 gene mutations).

log-transformed lamp2 concentrations were found for both diagnostic group ($p = 0.002$) and gender ($p = 0.042$). Table 2 shows additional details of the pairwise comparisons between diagnostic groups using Tukey-Kramer multiple comparison procedures. Female LRRK2 PD patients had significantly lower mean log-transformed lamp2 levels than the LRRK2 CTL ($p = 0.002$) and CTL ($p = 0.005$) groups. No other pairwise comparisons were statistically significant, although the p-value for the comparison of log-transformed lamp2 levels between LRRK2 PD females and sPD patients of either gender was 0.06.

2.4. Associations between lamp2 concentrations and age, oxidative stress markers, PD progression, and PD duration

Lamp2 levels in CSF were weakly correlated with age in all diagnostic groups (range of rho values: −0.15 to 0.22) and with 8-OHdG and 8-ISO in both control groups (range of rho values: −0.22 to 0.18). Correlations between lamp2 and the oxidative stress markers in the two PD groups were inconsistent, but three of the four correlations were ≥0.30 (rho values for sPD patients: lamp2 vs. 8-OHdG = 0.30, lamp2 vs. 8-ISO = 0.37; rho values for LRRK2 PD patients: lamp2 vs. 8-OHdG = 0.43, lamp2 vs. 8-ISO = 0.02). Lamp2 did not appear to be correlated with UPDRS scores or disease duration in either PD group (range of rho values: −0.27 to 0.10).

Table 1
Demographic and clinical characteristics for diagnostic groups. The groups were well matched for age. The LRRK2 PD group had a smaller proportion of males than the other groups. [a]UPDRS scores and duration of clinical disease were unavailable for one LRRK2 PD patient. (sPD = sporadic Parkinson's disease patients; LRRK2 PD = Parkinson's disease patients with LRRK2 mutations; CTL = healthy control subjects without known PD-associated mutations; LRRK2 CTL = healthy control subjects with LRRK2 mutations; UPDRS = Unified Parkinson's Disease Rating Scale; NA = not applicable).

Variable	sPD (n = 31)	LRRK2 PD (n = 19–20)[a]	CTL (n = 27)	LRRK CTL (n = 30)
Age, yrs (mean)	60.5	61.8	61.5	62.3
SD	7.9	10.3	9.3	10.7
% Males	48	20	37	40
UPDRS Total scores (median)	27	25	0	0
Range	16–55	20–75	0–0	0–7
UPDRS Part 3 scores (median)	20	19	0	0
Range	12–39	13–49	0–0	0–7
Clinical duration, yrs (median)	3	5	NA	NA
Range	<1–25	<1–21		