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DOI: 10.1002/ana.25523

Reply to "Serum Neurofilaments as Candidate Biomarkers of Natalizumab Progressive Multifocal Leukoencephalopathy"

Gloria Dalla Costa, MD,[1] Vittorio Martinelli, MD,[1] Roberto Furlan, MD, PhD,[2] and Giancarlo Comi, MD[1]

We read with interest the letter by Loonstra and colleagues in response to our recently published article on the role of serum neurofilament light chains (NfL) as early biomarkers of natalizumab-associated progressive multifocal leukoencephalopathy (PML) in patients with multiple sclerosis (MS).[1]

In our study, we found that at the onset of PML serum NfL were 10-fold higher than in the pre-PML condition and in natalizumab-treated or untreated MS patients. We therefore suggest that NfL may represent early and accessible markers of PML. The findings by Loonstra et al, with 4 additional cases with an increase in serum NfL at PML onset, support our hypothesis and further explore the dynamics of these changes. All our patients had a diagnosis of definite PML when we observed the increase in NfL, as demonstrated by JC viral DNA detection associated with the appropriate clinical and imaging features.[2] Loonstra and colleagues showed that also in asymptomatic patients fulfilling the criteria for probable PML,[2] NfL were already increased, and that in 2 patients the change in NfL commenced even prior to any other alert of a possible PML. It is noteworthy also that they validated our results by measuring serum NfL levels using Simoa technology, known to be a more accurate method than the electrochemiluminescence assay we used.[3] Furthermore, their observation of an almost perfect linear correlation between serum NfL and PML lesion volume is critical, as it means that serum NfL reflect brain damage and mirror MRI changes even in the case of small lesions, as occurs in the first phases of PML. These new findings further support the idea that NfL may represent a convenient tool to monitor patients at risk of PML and the need to immediately validate in a larger number of patients the value of longitudinal NfL changes as early signs of PML. Serum NfL measurement represents an additional value to conventional monitoring strategy for PML and may limit the burden of frequent MRI and the impact of this severe complication on patients' lives.

Potential Conflicts of Interest

G.D.C. reports no disclosures. V.M., R.F., and G.C received speaker's fees, consulting fees, or grant support from Biogen, which manufactures a drug used in the study.

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DOI: 10.1002/ana.25526

Mitochondrial DNA Deletions Discriminate Affected from Unaffected *LRRK2* Mutation Carriers

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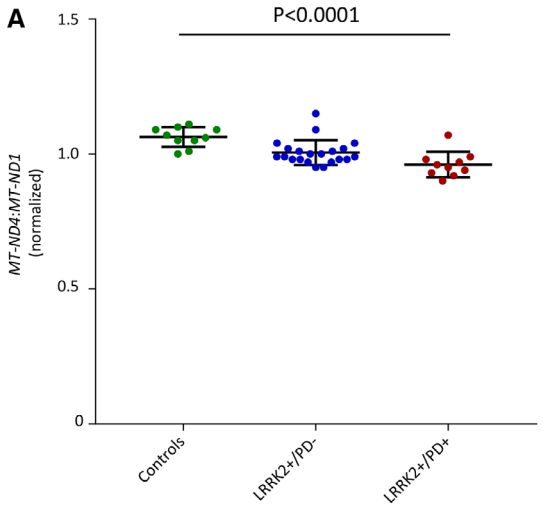
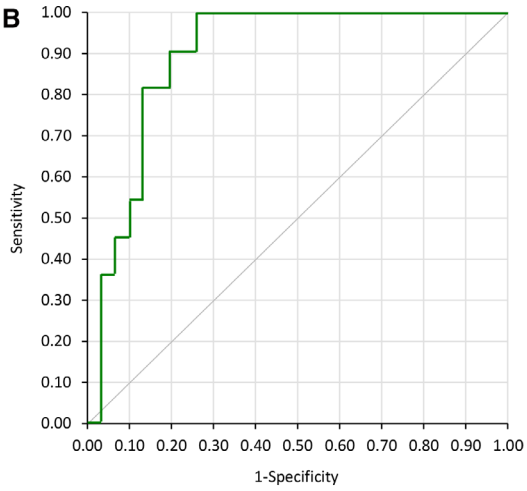
In a recently published article in the *Annals of Neurology*, Bakshi and colleagues determined the concentration of the antioxidant urate in serum samples from ~1,500 individuals with or without *LRRK2* mutations that were affected or unaffected by Parkinson disease (PD).[1] In 3 independent cohorts, they detected significantly lower levels of urate in manifesting ($LRRK2^+/PD^+$) compared to nonmanifesting ($LRRK2^+/PD^-$) *LRRK2* mutation carriers and speculated that the elevated PD risk in the $LRRK2^+/PD^+$ group may be due to increased LRRK2 activity,[2] which can interfere with urate-sensitive pathways including Nrf2 antioxidant signaling.[1] There is evidence that Nrf2 counteracts mitochondrial damage by triggering the expression of mitochondrial transcription factor A (TFAM).[3] TFAM functions as a mitochondrial transcription factor, but the protein is equally involved in mitochondrial DNA (mtDNA) replication and packaging of the mitochondrial genome into nucleoids.[4]

In search of a penetrance biomarker for LRRK2-associated PD, we explored the link between mtDNA integrity and disease progression. Employing a high-throughput multiplex real-time polymerase chain reaction assay,[4] we assessed the levels of mitochondrial major arc deletions in fibroblasts from manifesting ($LRRK2^+/PD^+$; n = 10, mean age ± standard deviation [SD] =

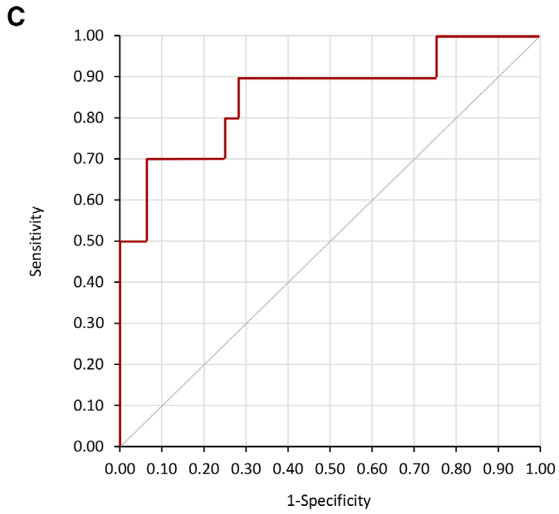
Correction added on November 25, 2019 after first online publication: the copyright line has been revised.



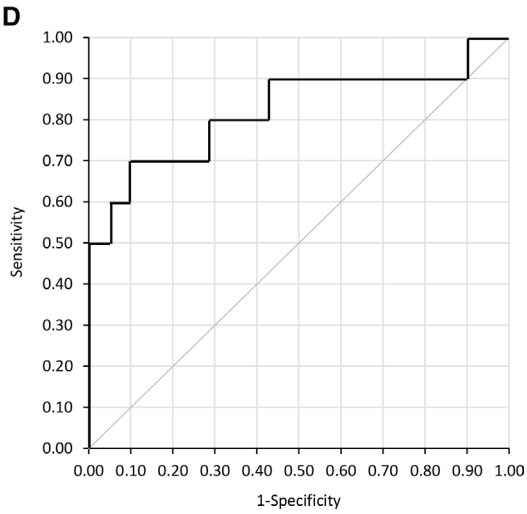
Status	Mean *MT-ND4:MT-ND1* (95% CI)
Control	1.063 (1.037-1.089)
LRRK2+/PD-	1.005 (0.984-1.026)
LRRK2+/PD+	0.961 (0.927-0.995)



Comparison	AUC (95% CI)
Controls vs (LRRK2+/PD- & LRRK2+/PD+)	0.90 (0.76-1.03)



Comparison	AUC (95% CI)
LRRK2+/PD+ vs (LRRK2+/PD- & controls)	0.86 (0.70-1.01)



Comparison	AUC (95% CI)
LRRK2+/PD+ vs LRRK2+/PD-	0.82 (0.65-1.0)

FIGURE: Analysis of mtDNA major arc deletions in controls, and nonmanifesting (LRRK2$^+$/PD$^-$) and manifesting carriers (LRRK2$^+$/PD$^+$) of the LRRK2 G2019S mutation. (A) *MT-ND4:MT-ND1* ratios differed significantly between the control, LRRK2$^+$/PD$^-$, and LRRK2$^+$/PD$^+$ groups, as determined by 1-way analysis of variance ($p < 0.0001$). Dots indicate mean values per person derived from 3 independent experiments. In addition, group mean values with standard deviations and 95% confidence intervals (CIs) are shown. A logistic regression model was used to predict the outcome of Parkinson disease (PD) status in *LRRK2* mutation carriers with *MT-ND4:MT-ND1* ratios, adjusted by age (LRRK2$^+$/PD$^-$ vs LRRK2$^+$/PD$^+$, odds ratio = 2.40, 95% CI = 1.05–5.16, $p = 0.014$). (B–D) Receiver operating characteristic curves. Areas under the curve (AUCs) and 95% CI are given for different group comparisons as indicated in the tables.

66.0 ± 12.5 years) and nonmanifesting carriers (LRRK2$^+$/PD$^-$; n = 21, mean age ± SD = 58.5 ± 15.4 years) of the G2019S mutation and healthy mutation-negative controls (n = 10, mean age ± SD = 58.3 ± 14.5 years). mtDNA deletion levels were derived from the *MT-ND4:MT-ND1* ratio. Analysis of variance (ANOVA) testing, regression models, and investigation of sensitivity with receiver operating characteristic (ROC) curves were constructed using statistical analysis software (JMP 14; SAS Institute, Cary, NC). The mean values for *MT-ND4:MT-ND1* differed significantly between the 3 groups (1-way ANOVA: $F = 13.26$, $p < 0.0001$), with the highest deletion levels in manifesting G2019S mutation carriers (significance levels after Bonferroni correction for multiple testing: controls vs LRRK2$^+$/PD$^-$, $p = 0.0047$; controls vs LRRK2$^+$/PD$^+$, $p < 0.0001$; LRRK2$^+$/

PD$^-$ vs LRRK2$^+$/PD$^+$, p = 0.0355). The levels of somatic mtDNA deletions in *LRRK2* mutation carriers were associated with PD status even after adjusting for age in a logistic regression model, where the disease status is the dependent variable and the covariates are the *MT-ND4*:*MT-ND1* ratio and age (LRRK2$^+$/PD$^+$ vs LRRK2$^+$/PD$^-$, odds ratio = 2.40, 95% confidence interval = 1.05–5.16, p = 0.014). The sensitivity of the *MT-ND4*:*MT-ND1* ratio as a biological marker was assessed using covariant-adjusted ROC analysis. The resulting areas under the curves (>0.75) indicated good discrimination for all investigated comparisons: (1) controls versus all individuals with LRRK2 G2019S, (2) LRRK2$^+$/PD$^+$ versus all unaffected individuals, and (3) LRRK2$^+$/PD$^+$ versus LRRK2$^+$/PD$^-$ (Fig).

Increased levels of reactive oxygen species resulting from reduced urate concentrations in manifesting G2019S mutation carriers may also be the cause of the mtDNA phenotype[5] observed in this study. Our finding of increased mtDNA deletions in LRRK2$^+$/PD$^+$ compared to LRRK2$^+$/PD$^-$ individuals supports a link between LRRK2 kinase activity,[2] urate-mediated Nrf2 signaling, and oxidative stress in the progression of LRRK2-associated PD.

Acknowledgment

A.G. was supported by grants from the Luxembourg National Research Fund (FNR) in the ATTRACT (Model IPD, FNR9631103) and INTER programs (ProtectMove, FNR11250962). A.G., P.S., I.R.K., and C.K. received funding from the German Research Foundation (DFG) within the framework of the research unit ProtectMove (GR 3731/5-1, SE 2608/2-1, KO 2250/7-1, FOR 2488/1). J.T. is an Alexander von Humboldt fellow and receives funding from the Canadian Institutes of Health Research and the Joachim Herz Foundation. This project was supported by the high-throughput/high-content screening platform at the Luxembourg Center for Systems Biomedicine (LCSB).

Potential Conflicts of Interest

Nothing to report.

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DOI: 10.1002/ana.25510

Reply to "Mitochondrial DNA Deletions Discriminate Affected from Unaffected *LRRK2* Mutation Carriers"

Rachit Bakshi, PhD,[1,2] Eric A. Macklin, PhD,[2,3] and Michael A. Schwarzschild, MD, PhD[1,2]

We thank Ouzren et al for their interest and insightful commentary on our article.[1] They report increased mitochondrial DNA (mtDNA) deletions in fibroblasts derived from *LRRK2* Parkinson disease (PD) patients compared to nonmanifesting *LRRK2* mutation carriers and propose an interesting mechanism potentially linking urate levels, Nrf2 signaling, and mtDNA integrity.

Other groups have also reported higher mtDNA damage in LRRK2 in vitro and in vivo models.[2,3] We agree with the authors that their results support mtDNA damage as a promising biomarker of PD risk and progression in *LRRK2* patients and suggest additional aspects of *LRRK2* PD that are worthy of further investigation. We are currently exploring mitochondrial dysfunction in induced pluripotent stem cells derived from *LRRK2*$^+$ and *LRRK2*$^-$ PD patients and unaffected controls.[4]

Other measures of mtDNA integrity may also highlight unique features of *LRRK2* PD or suggest *LRRK2*-specific pathophysiology. Recent studies have shown a decreased amount of cell-free mtDNA in cerebrospinal fluid (CSF) of patients affected by Alzheimer disease and PD.[5,6] However, Podlesniy et al[7] reported a higher concentration of cell-free mtDNA in CSF of *LRRK2*$^+$ PD, compared with nonmanifesting carriers and idiopathic PD patients. Whether cell-free mtDNA correlates with intracellular mitochondrial dysfunction is unclear. Further studies in large datasets like the LRRK2 Cohort Consortium and the Parkinson's Progression Markers Initiative will be helpful to understand the role of cell-free mtDNA as a biomarker of neurodegeneration or disease progression in different forms of PD.

Potential Conflicts of Interest

Nothing to report.