Reply to "Urinary α-Synuclein for Parkinson's Detection: Key Limitations and Future Directions"

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Dear Editor,

We thank Dr. Ma, Dr. Deng, and Dr. Liu for their thoughtful and constructive comments on our recent publication investigating elevated urinary α-synuclein aggregate levels in patients with isolated rapid eye movement (REM) sleep behavior disorder (iRBD) and Parkinson's disease (PD).[1] We appreciate the opportunity to respond to the concerns raised.

Regarding the timing of urine collection, samples in our study were obtained between 8:00 AM and 4:00 PM. We acknowledge that diurnal variation can influence the concentration of urinary biomarkers, including α-synuclein aggregates. However, the specific impact of collection time on α-synuclein levels in concentrated urine remains to be fully elucidated. We agree that future studies may benefit from either standardizing the timing of collection or stratifying analyses based on collection time to improve reproducibility and comparability.

We also concur with the observation that α-synuclein levels may fluctuate over the course of disease progression, reflecting the heterogeneous stages of PD and iRBD among patients. In response to this important point, we are about to undertake a longitudinal study with serial urine samples collected from patients and healthy controls. This will allow us to investigate temporal changes in urinary α-synuclein levels and evaluate the impact of potential external modulators.

With regard to demographic matching, we are mindful of the age and sex differences between patient and control cohorts. As noted in our study, we performed a subgroup analysis including only healthy controls over the age of 50 (median age 64.4 ± 7.6 years), which closely matched the median ages of the PD (64.3 ± 9.7 years) and iRBD (66.3 ± 6.4 years) groups.[1] The significant differences in urinary α-synuclein fibril concentrations remained consistent in these analyses. Additionally, when stratified by sex—comparing only male PD and iRBD patients to male healthy controls (Fig A), and only female patients to female controls (Fig B)—the statistical significance of observed differences was preserved. We acknowledge, however, that prospective recruitment strategies aimed at minimizing age and sex disparities, such as stratified random sampling or tighter age-matching protocols, may reduce residual confounding in future investigations. The use of multivariable statistical models incorporating age and sex as covariates is another valuable approach to adjust for demographic differences and strengthen the robustness of biomarker comparisons.

Last, we note that the primary aim of our study was to quantify urinary α-synuclein aggregates and assess their diagnostic potential in synucleinopathies. While the inclusion of additional biomarkers may

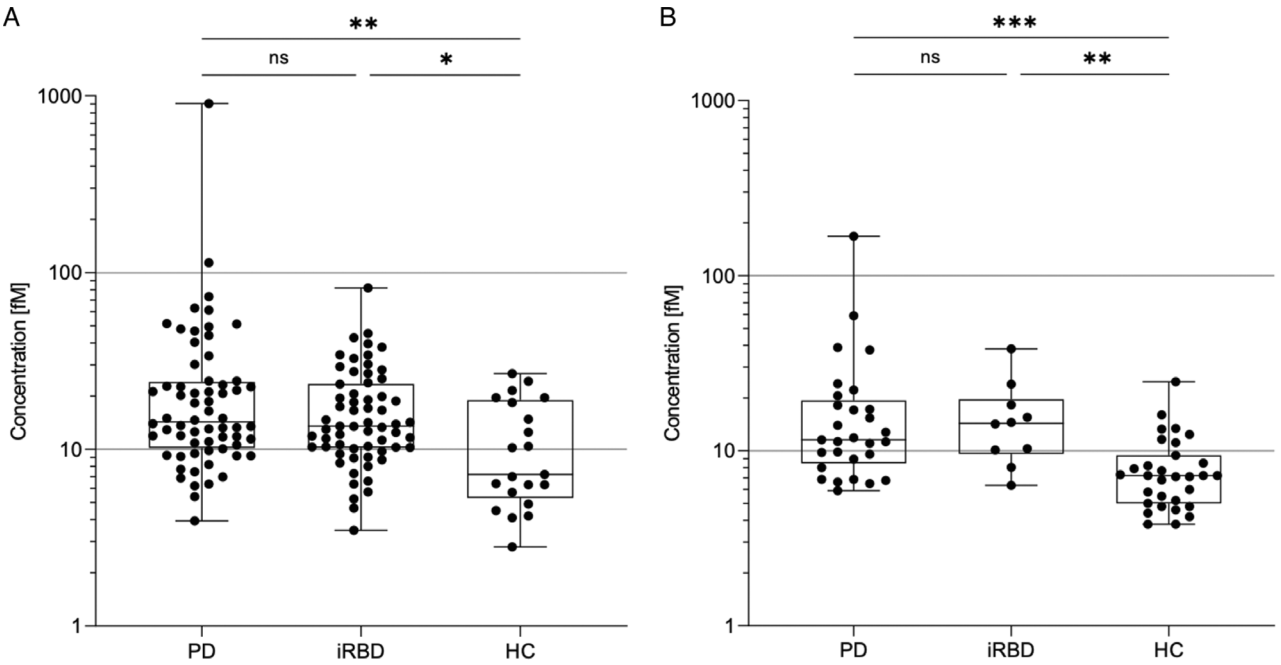

FIGURE: Concentration of α-synuclein aggregates in urine samples stratified by gender and cohort. (A) The median concentration of α-synuclein aggregates in 10× concentrated urine of male patients with Parkinson's disease (PD) (14.3 fM, $^{**}p < 0.01$, n = 64) and isolated rapid eye movement sleep behavior disorder (iRBD) (13.5 fM, $^{*}p < 0.05$, n = 62) was significantly higher than in the urine of the male healthy controls (HC) (7.2 fM, n = 21). (B) The median concentration of α-synuclein aggregates in 10× concentrated urine of female patients with PD (11.5 fM, $^{***}p < 0.001$, n = 29) and iRBD (14.4 fM, $^{**}p < 0.01$, n = 10) was significantly higher than in the urine of the female HCs (7.2 fM, n = 31). Surface-based fluorescence intensity distribution analysis (sFIDA) readouts were converted to concentrations using sFIDAta version 2.2.7. Significance was determined using GraphPad Prism version 10.4.0 and the Kruskal–Wallis test together with Dunn's test as a post hoc test (ns = non-significant).

further enhance diagnostic precision, such analyses were beyond the scope of the current work and are planned for future studies.

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Author Contributions

Laura Müller: Methodology; investigation; validation; formal analysis; data curation; software; visualization. **Pelin Özdüzenciler:** Writing – review and editing. **Charlotte Schedlich-Teufer:** Writing – review and editing. **Aline Seger:** Writing – review and editing. **Hannah Jergas:** Writing – review and editing. **Gereon R. Fink:** Writing – review and editing. **Dieter Willbold:** Writing – review and editing. **Michael Sommerauer:** Writing – review and editing; conceptualization; project administration; resources; funding acquisition. **Michael T. Barbe:** Writing – review and editing; conceptualization; project administration; resources; funding acquisition. **Gültekin Tamgüney:** Conceptualization; writing – review and editing; writing – original draft; supervision; project administration; funding acquisition.

Potential Conflicts of Interest

D.W. is co-founder of attyloid, which is commercializing the sFIDA assay. All other authors have nothing to report.

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From Data to Decision: Reinventing Hypoxic–Ischemic Encephalopathy Prognosis with Magnetic Resonance Imaging and Machine Learning

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To the Editor,

Neonatal hypoxic–ischemic encephalopathy (HIE) is a severe complication of perinatal asphyxia, affecting survival and neurodevelopment.[1] Predicting long-term outcomes remains challenging. Although magnetic resonance imaging (MRI) detects brain injuries, its reliance on expert interpretation limits quantification of mild damage and early intervention accuracy.[2] With artificial intelligence (AI) and medical imaging advances, machine learning is increasingly used to improve neonatal neuroprognostication through data-driven methods.

Lewis and his team[3] applied machine learning to MRI data to predict neurodevelopmental outcomes in neonates receiving therapeutic hypothermia. They standardized MRI preprocessing and used an Elastic-Net regression model to estimate cognitive, language, and motor outcomes based on Bayley-III assessments. The results showed that MRI-based features outperformed demographic and laboratory data, identifying key imaging markers. This study developed an automated HIE prognostication model, strengthening imaging-based prediction and advancing AI integration in neonatal neurology. Further research is needed to refine its clinical application.

Elastic-Net regression struggles to capture the nonlinear patterns in MRI data. While it enhances feature selection and stability, HIE-related brain injuries involve complex, nonlinear changes in deep gray matter, white matter microstructure, and texture. Elastic-Net may not fully capture these relationships. Studies show that nonlinear models like Extreme Gradient Boosting and Convolutional Neural Networks often outperform linear methods in medical imaging analysis.[4,5] Future research should explore more flexible models to improve predictive accuracy.

The accuracy of MRI segmentation remains unverified, potentially affecting the model's predictive performance. The authors used ANTs for standardization and a CNN for brain mask generation, but did not provide Dice similarity coefficient (DSC) evaluation or manual segmentation comparison. This could introduce measurement bias in key brain regions, impacting HIE prognosis accuracy. Future studies should assess DSC consistency, validate segmentation robustness on