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Short Communication

Respiratory partitioning reveals peripheral bioenergetic heterogeneity in amyotrophic lateral sclerosis



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Introduction

Amyotrophic lateral sclerosis (ALS) exhibits marked clinical heterogeneity, and previous studies have reported mitochondrial abnormalities in circulating blood cells [1,2]. Oxygen flux alone may not capture how ongoing respiration relates to available electron-transfer (ET) capacity. We examined whether integrating protein-normalized fluxes with respiratory partitioning identifies peripheral bioenergetic profiles in ALS and whether these profiles relate to clinical involvement.

Methods

This cross-sectional case–control study included 63 patients with ALS and 61 age- and sex-matched controls. Freshly isolated peripheral blood mononuclear cells (PBMCs) were measured at 37 °C in an O2k high-resolution respirometer using a substrate–uncoupler–inhibitor titration protocol. Routine respiration (*R*), pyruvate-supported respiration (ce1P), oligomycin-associated LEAK respiration (*L*), ET capacity (*E*), and succinate-supported states before and after plasma-membrane permeabilization (1Dig) were recorded. Two technical chambers per participant were normalized separately to protein content and averaged; participants were the unit of analysis. Age- and sex-adjusted case–control models were applied to ten flux and ratio outcomes, with Benjamini–Hochberg correction. Within ALS, six standardized respiratory features were used for sparse k-means clustering. Clinical variables were excluded from clustering, and cluster stability was assessed in 500 bootstrap resamples.

Results and discussion

ce1P and post-permeabilization 1Dig were lower in ALS than in controls (false discovery rate-adjusted P = 0.043 and P < 0.001, respectively). Routine respiration, *L*, and *E* did not differ significantly between groups. The two-cluster (K2) solution separated 40 and 23 patients into profiles with opposing patterns of fractional respiratory engagement and relative ET reserve; mean bootstrap Jaccard similarities were 0.871 and 0.819. The three-cluster (K3) solution provided additional exploratory resolution but was less stable, with K3 C2 dissolving in 58 % of resamples. K3 C3 (n = 20; King's stage available for 19) combined greater fractional engagement, lower reserve efficiency, and lower *E* relative to controls. Compared with K3 C1, this profile was associated with higher King's stage in an age- and sex-adjusted proportional-odds model (odds ratio 4.95, nominal 95 % confidence interval 1.50–16.28; Holm-adjusted P = 0.0255). ALS Functional Rating Scale–Revised scores and estimated progression rates did not differ significantly between clusters. Joint assessment of flux magnitude and its relationship to capacity may reveal patient-level respiratory heterogeneity that pooled comparisons do not resolve [3]. The stage association is exploratory and cross-sectional; it does not establish prognostic value or a cell-intrinsic mitochondrial defect. Independent longitudinal studies with PBMC subset phenotyping are needed to determine whether these profiles persist and to clarify the contributions of cell composition and within-cell remodeling [4–6].

Conflicts of interest: The authors declare no conflicts of interest.

Author contributions: Investigation: NH, JS. Data curation: NH, LCh. Formal analysis: NH, MGr,. Methodology and software: MGr. Validation: AIV, MS. Visualization: NH, IP. Resources: MKop, EK, LCh. Conceptualization, funding, supervision, and project administration: MK.

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