Lipidomic analysis of patients with systemic sclerosis and idiopathic inflammatory myopathies

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1 INTRODUCTION AND AIMS

Systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) are rare autoimmune connective tissue diseases associated with chronic inflammation, immune dysregulation, and progressive fibrosis. Cardiovascular (CV) involvement is a major contributor to morbidity and mortality but is often underestimated. Traditional CV risk scores like SCORE/SCORE2 may not fully capture risk in these populations.

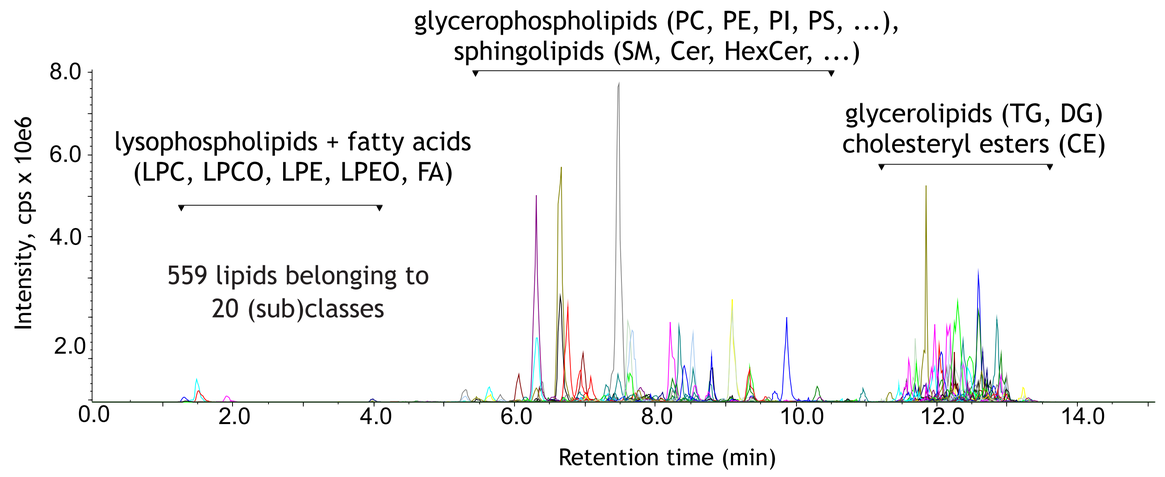
TThis study aimed to compare plasma lipid profiles in patients with SSc and IIM versus age-/sex-matched healthy controls (HC), all without manifest CV disease, and to evaluate the applicability of the Coronary Event Risk Test (CERT) score for CV risk estimation in these diseases.

2 PATIENT COHORT

A total of 390 samples were subjected to lipidomic analysis. Plasma samples were collected from patients with SSc (n = 100) and IIM (n = 90) for the study. Together with the patient samples, 200 samples from healthy controls were analysed. The samples were provided by the RU biological material bank.

3 TARGETED LIPIDOMIC ANALYSIS OF PLASMA

- Lipids were extracted using 100% isopropanol containing deuterated internal standards.
- Deproteination was performed overnight at -20 °C.
- After centrifugation, the obtained extract was subjected to LC-MS/MS analysis.
- **LC-MS/MS:**
  RPLC (C8, BEH, 1.7 µm, 2.1 mm × 100 mm); MS/MS (QqQ, +/- MRM)
  MFA: AcN:H_2O (3:2, v/v) + 10mM CH_3COONH_4
  MFB: IPA:AcN (9:1, v/v) + 10mM CH_3COONH_4



4 RESULTS

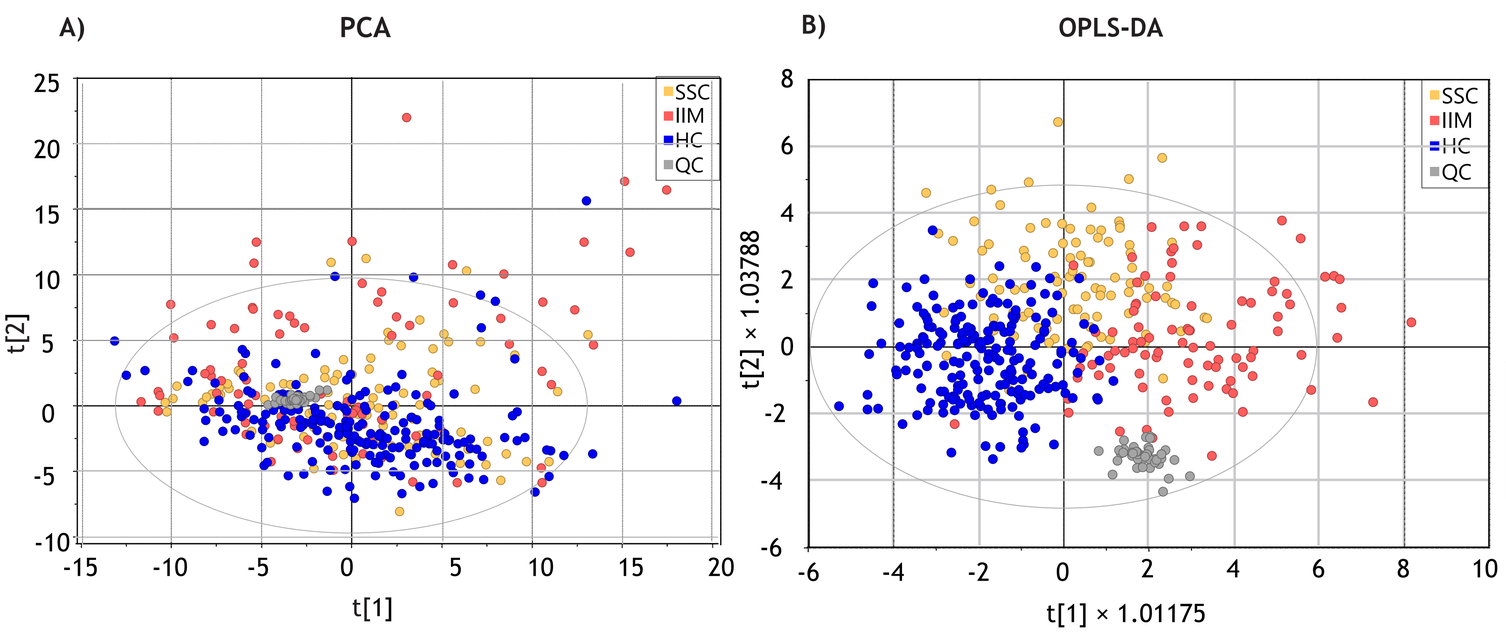


Figure 1: Multivariate statistical analysis. Principal Component Analysis (PCA, **A**) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA, **B**) partially distinguishing SSc (yellow) and IIM (orange) from healthy controls (blue). Close clustering of quality control (QC) sample (grey) indicates reproducibility of analyses.

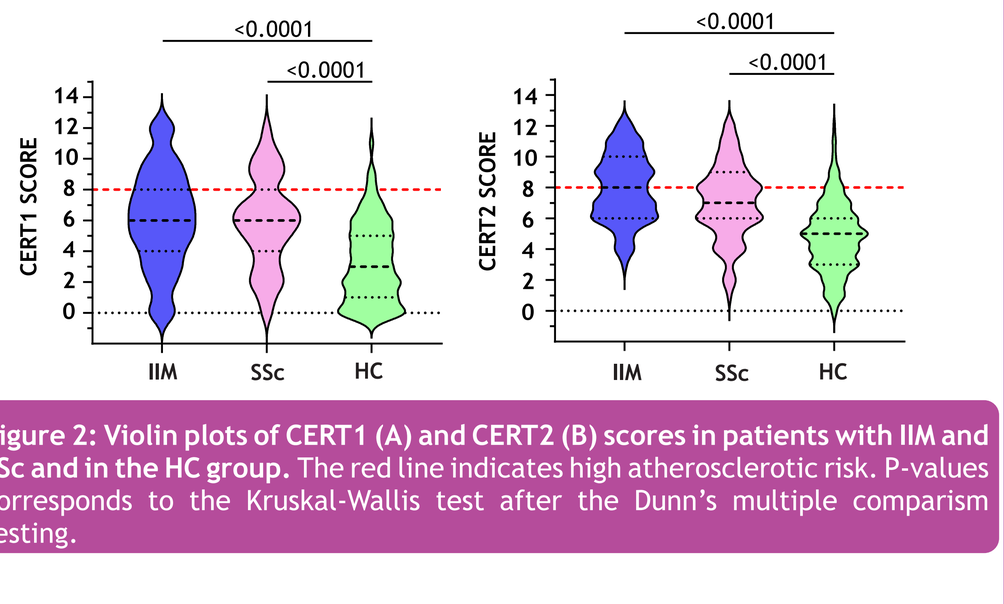
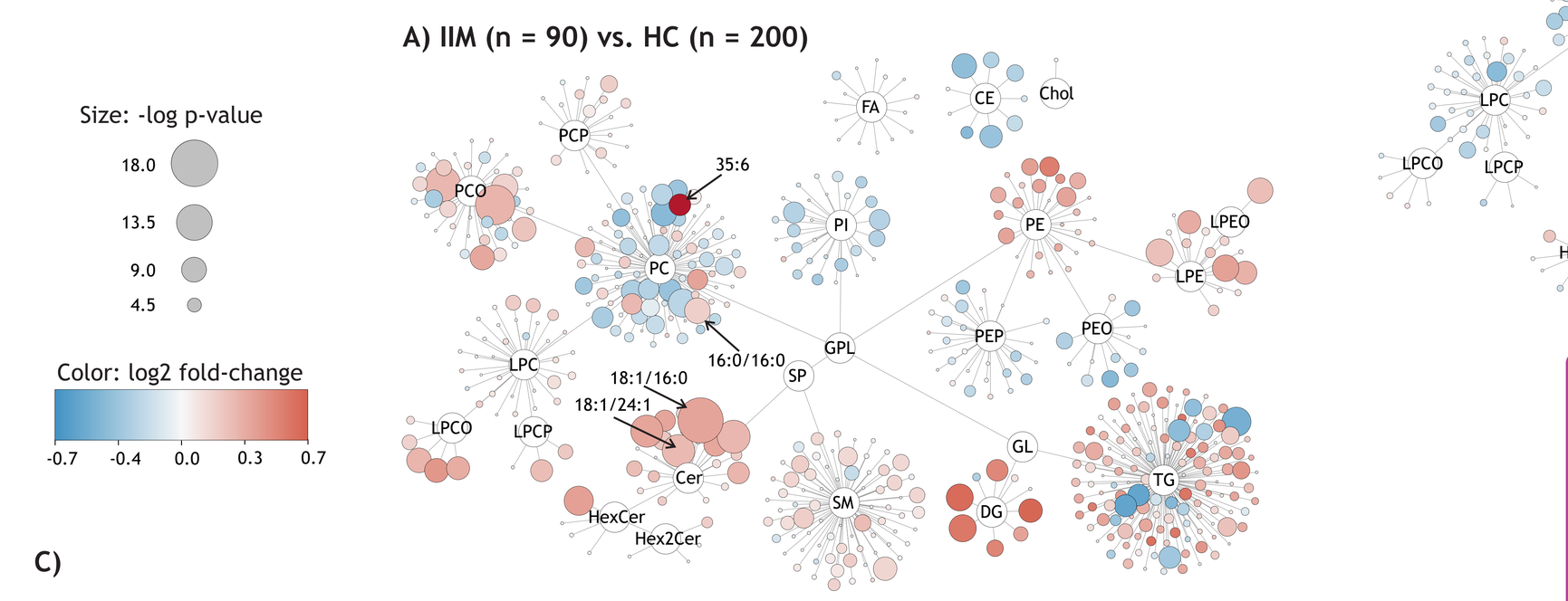


Figure 2: Violin plots of CERT1 (A) and CERT2 (B) scores in patients with IIM and SSc and in the HC group. The red line indicates high atherosclerotic risk. P-values corresponds to the Kruskal-Wallis test after the Dunn's multiple comparism testing.



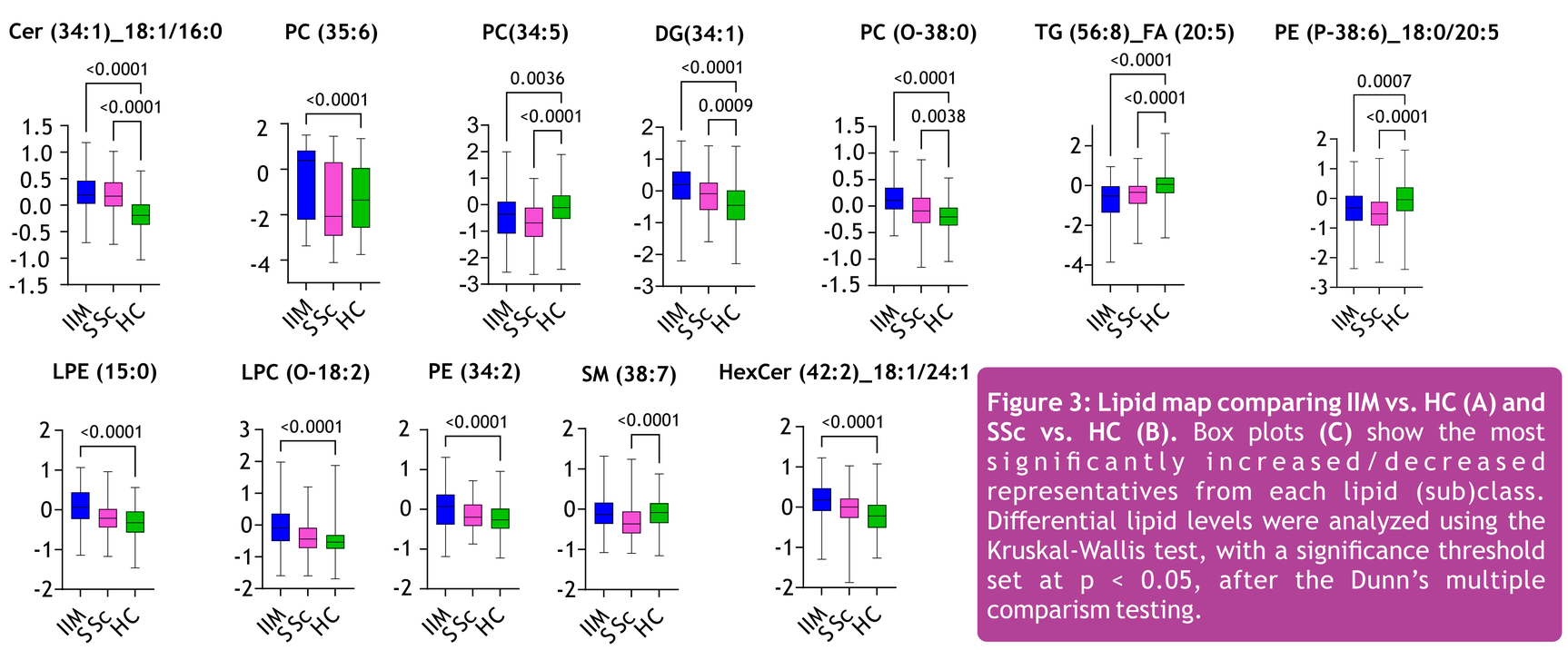


Figure 3: Lipid map comparing IIM vs. HC (A) and SSc vs. HC (B). Box plots **(C)** show the most significantly increased/decreased representatives from each lipid (sub)class. Differential lipid levels were analyzed using the Kruskal-Wallis test, with a significance threshold set at $p < 0.05$, after the Dunn's multiple comparism testing.

5 CONCLUSIONS

- This study demonstrated distinct lipidomic alterations in patients with SSc and IIM compared to healthy controls. Multivariate analyses using PCA (**Fig. 1A**) and OPLS-DA (**Fig. 1B**) revealed partially overlapping but separable profiles, indicating the presence of disease-specific metabolic signatures.
- CERT1 (**Fig. 2A**) and CERT2 (**Fig. 2B**) scores were significantly elevated in both IIM and SSc patients compared to healthy individuals. These findings point to an increased atherosclerotic risk in both disease groups, which may not be fully reflected by traditional cardiovascular risk assessment tools such as SCORE or SCORE2. This highlights the potential clinical value of CERT scores in autoimmune settings.
- Lipid class-level comparisons (**Fig. 3A, 3B**) showed increased levels of diacylglycerols (DG), lysophosphatidylethanolamines (LPE/O), lysophosphatidylcholines (LPCO/P), and phosphatidylethanolamines (PE) in IIM patients. In contrast, SSc patients exhibited broader decreases, particularly in phosphatidylcholines (PC), lysophosphatidylcholines (LPC), sphingomyelins (SM), plasmalogens (PEP), and ether-linked PE (PEO), suggesting different mechanisms of lipid dysregulation between these diseases (**Fig. 3C**).