

GC/MS-based metabolomics detects metabolic alterations in serum from SLE patients

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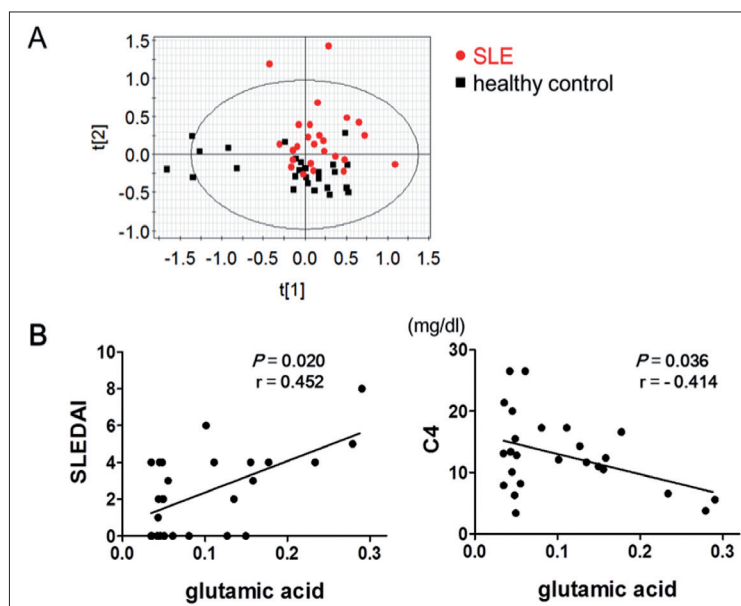
Metabolomics, or metabolome analysis, is the comprehensive identification and quantification of all the low-molecular-weight metabolites in a biological sample. Because metabolism closely influences the organism's phenotype, the characteristics of a disease are thought to more closely reflect alterations in the levels of metabolites than changes in gene or protein expression. Two recent studies revealed the metabolic disturbances associated with systemic lupus erythematosus (SLE) using metabolomics technologies such as nuclear magnetic resonance (NMR) analysis, gas chromatography/mass spectrometry (GC/MS), and liquid chromatography/mass spectrometry (LC/MS) (1, 2). Among these methods, GC/MS generates superior data with high reproducibility, repeatability, and robustness, and has a more abundant database for multiple metabolites (e.g. National Institute of Standards and Technology (NIST) MS database) than do other technologies (3). Thus we hypothesized that GC/MS-based metabolomics could detect metabolic alterations in serum from SLE patients and identify candidates for sensitive metabolic biomarkers which are useful for diagnosing and/or monitoring the disease.

Serum samples were obtained in the morning from fasting SLE patients ($n=26$, 38.8 ± 10.1 y.o.) and healthy volunteers ($n=26$, 39.5 ± 3.1 y.o.). All SLE patients were women, and taking prednisolone (3–25 mg daily) with or without immunosuppressive agents. Serum metabolite profiling was performed by GC/MS, and 62 metabolites were detected in the subjects' sera. The levels of 25 of the 62 serum metabolites were significantly different in SLE patients compared to healthy volunteers ($p < 0.05$).

We then performed principal component analysis (PCA) based on the 62 metabolites, and the two-dimensional (2D)-PCA scores plot showed a distinct clustering of the two groups (Fig. 1A). The corresponding 2D-PCA loadings plot and the 2D-scores plot for partial least squares-discriminant analysis (PLS-DA) loadings plot show the metabolites characterising the SLE patients were glutamic acid, glycerol, urea, phosphate, and tyrosine.

In order to establish whether similar metabolic perturbations accompany other systemic autoimmune diseases, we performed serum metabolomics for rheumatoid arthritis (RA), another prototypic systemic autoimmune disease ($n=32$), and compared the serum metabolite levels with healthy subjects and SLE. There was a large difference in serum metabolite levels between

Fig. 1.



SLE and RA, and a number of metabolites showed opposite changes between SLE and RA (data not shown).

We next investigated the correlation between the serum levels of metabolites and clinical and serological markers of disease activity in SLE. Of 25 metabolites that were significantly changed in SLE, the serum level of glutamic acid was significantly correlated with the SLE disease activity index (SLEDAI) score and serum levels of C4 (Fig. 1B).

We previously found intra- and inter-day variance in the levels of some amino acids, probably due to the effects of diet and/or daily activity (4, 5). Therefore, serum samples were obtained from all subjects after overnight fasting in this study.

Medications may also affect the serum levels of some metabolites. Consistent with a previous report, the serum level of phenylalanine showed significant association with the dose of corticosteroid (2). Beside this, the other metabolic changes described in this paper showed no correlation with the medications the patients were on.

Our present findings suggest that the pathogenesis of SLE may be accompanied by variations in the serum levels of low-molecular-weight metabolites, which supports the potential for using GC/MS-based metabolomics as a diagnostic and monitoring tool for SLE, although the populations used in this study were not large enough, and larger validation studies are therefore needed to verify its practical utility. Moreover, to arrive at a complete assessment of the usefulness of metabolic perturbations in the diagnosis of SLE, any future study should include other groups of patients with a diagnosis other than RA, such as Sjögren's syndrome, systemic sclerosis, polymyositis/dermatomyositis, and vasculitis.

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