UNTARGETED METABOLOMICS OF EARLY VASCULAR AGING IN HYPERTENSIVE PATIENTS

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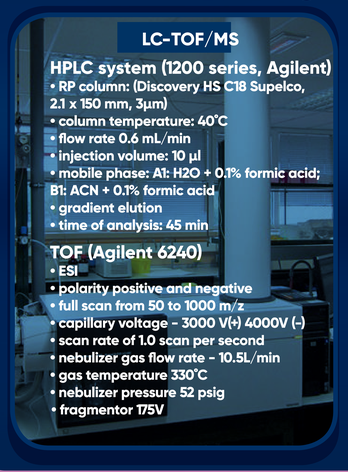
Introduction

Arterial stiffening is a hallmark of early vascular aging (EVA) syndrome and an independent predictor of cardiovascular morbidity and mortality. Early vascular ageing (EVA), reflected by increased pulse wave velocity, has been associated with premature CV diseases manifestation including coronary heart disease and stroke. Potential mechanisms underlying EVA process include arterial stiffening and impaired endothelial function. Small-molecule metabolites may reflect pathological state and serve as novel markers of EVA state.

Aim of the study

In this case–control study we sought to identify plasma metabolites associated with EVA syndrome in the setting of hypertension. An untargeted metabolomic approach was used to identify plasma metabolites in an age-, BMI-, and sex-matched groups of EVA (n = 79) and non-EVA (n = 73) individuals with hypertension. Principal component analysis (PCA) was used to evaluate quality of analyses and general trends in the data. Hotelling's T2 range was used to detect potential outliers. Least Absolute Shrinkage and Selection Operator (LASSO) were used to select metabolites which contribute the most to recognition between non-EVA and EVA group. A reproducibility of the results was assessed with a resampled-based bootstrap procedure.

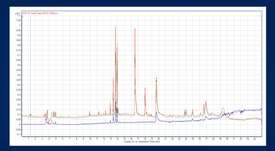
Plasma metabolic fingerprinting



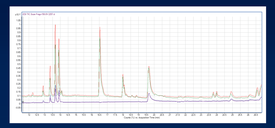
LC-TOF/MS

HPLC system (1200 series, Agilent)
- RP column: (Discovery HS C18 Supelco, 2.1 x 150 mm, 3µm)
- column temperature: 40°C
- flow rate 0.6 mL/min
- injection volume: 10 µl
- mobile phase: A1: H2O + 0.1% formic acid; B1: ACN + 0.1% formic acid
- gradient elution
- time of analysis: 45 min

TOF (Agilent 6240)
- ESI
- polarity positive and negative
- full scan from 50 to 1000 m/z
- capillary voltage - 3000 V(+) 4000V (-)
- scan rate of 1.0 scan per second
- nebulizer gas flow rate - 10.5L/min
- gas temperature 330°C
- nebulizer pressure 52 psig
- fragmentor 175V



LC-TOF/MS (+)



LC-TOF/MS (-)

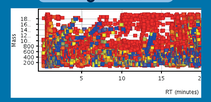
Data treatment

LC-TOF/MS data

Features Finding

MassHunter Qualitative Analysis

Alignment

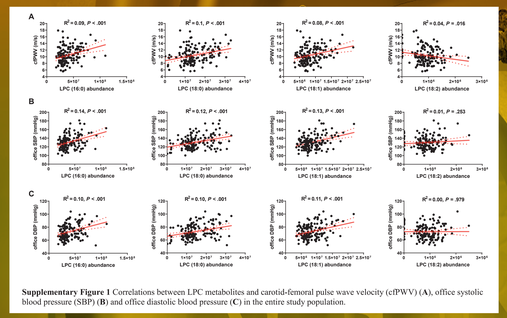


Mass Profiler Professional

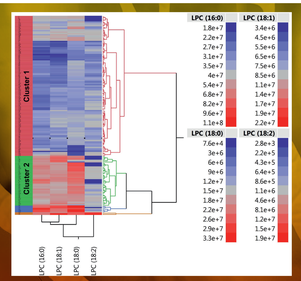
Filter by Frequency

90% in at least one of the group

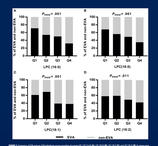
Biological interpretation

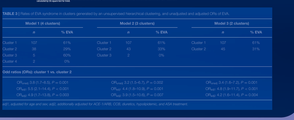


Supplementary Figure 1 Correlations between LPC metabolites and carotid-femoral pulse wave velocity (cfPWV) (**A**), office systolic blood pressure (SBP) (**B**) and office diastolic blood pressure (**C**) in the entire study population.



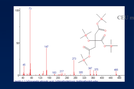
Group classification





Metabolite identification

- Accurate masses of features which represented statistically significant differences were searched for structure against the METLIN, KEGG, LIPIDMAPS, HMDB databases and CEU MASS mediator database
- Comparison of the structure of the proposed compound with the spectra obtained by LC-QTOF/MS fragmentation analyses confirms the identity of metabolites



Checking of quality of analysis

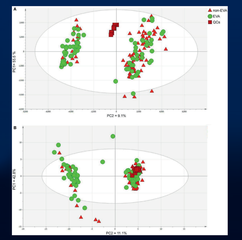


FIGURE 1 | PCA models for dataset after filtration obtained in positive (**A**) and negative (**B**) ion modes. PC1/2—principal component 1/2. Data were log transformed and Pareto or UV scaled in the case of positive and negative ionization modes, respectively. (**A**) PCs contribution (PC1 = 55.6 % and PC2 = 9.1%). (**B**) PCs contribution (PC1 = 42.8%, PC2 = 11.1%).

Statistical analysis

- All analyses were performed with "penalized" and "rcorr" package in R Core Team (2014) to fit the LASSO model and perform the correlation analysis, respectively. PCAmodeling and plotting were performed with the use of SIMCA software (version P13, Umetrics, Umeå, Sweden).
- An unsupervised hierarchical cluster analysis with Ward's method for defining distances between clusters was used to determine a metabolomic signature of EVA. Binary logistic regression was applied to calculate unadjusted and adjusted odds ratios (ORs), to assess the association between given clusters (profiles) and a risk of EVA.

Conclusions

- ✓ The hypertensive individuals were characterized by 4 down-regulated LPCs had 3.8 times higher risk of EVA compared to those with higher LPC levels (OR = 3.8, 95% CI 1.7–8.5, P < 0.001).
- ✓ Our results provide new insights into a metabolomic phenotype of vascular aging and warrants further investigation of negative association of LPCs with EVA status.
- ✓ This study suggests that LPCs are potential candidates to be considered for further evaluation and validation as predictors of EVA in patients with hypertension.
- ✓ The reproducibility of 4 selected metabolites was found to be within a relatively wide range (39–65%) which is likely a consequence of a relatively small sample size and variability present in the data.

Acknowledgements

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