Mäkinen, V. P., & Ala-Korpela, M. (2016). Metabolomics of aging requires large-scale longitudinal studies with replication. Proceedings of the National Academy of Sciences, 113(25), [E3470]. DOI: 10.1073/pnas.1607062113

Peer reviewed version

Metabolomics of aging requires large-scale longitudinal studies with replication

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Aging is a complex biological process with remarkable individual variation and manifests also as changes in systemic metabolism. Chaleckis and co-authors studied a set of blood metabolites in old and young individuals in a recent paper in the Journal (1). The work was thorough in the methodological aspects regarding the mass spectrometry of circulating molecules in plasma, red blood cells and whole blood. However, we find it alarming that the results and interpretations on the suggested age-related metabolic differences were not properly put into epidemiological context. We therefore draw attention to four key issues: i) metabolic heterogeneity of individuals, ii) cross-sectional vs longitudinal study design, iii) multiple statistical tests, and iv) independent biological replication.

Individuals within a population exhibit complex interactions of genetic, environmental and incidental factors that ultimately determine the metabolic snapshot obtainable via a blood sample. Typically, a few thousand individuals are needed for robust biomarker findings regarding complex outcomes (2). To this backdrop, it is regrettable to see causal and mechanistic claims being made on the basis of average differences between two groups, with only 15 individuals per group. Unfortunately, similar studies and claims still occur in metabolomics despite contrary experience from larger cohorts (3).

Comparisons at a single time point should be interpreted with extreme caution, since even large-scale cross-sectional studies may be severely confounded by lifestyle, socioeconomic factors, baseline health status and treatment effects (4). Consequently, cross-sectional studies typically fail to capture true age-related phenomena. This has been emphasized in the Journal by Belsky and co-authors based on their combined cross-sectional and longitudinal findings from a large population cohort; aging studies should incorporate longitudinal measures of biomarkers to track changes (5).

The explosion of high-throughput omics technologies has brought forward the essential role of robust statistics, particularly the management of multiple testing. Chaleckis and co-authors measured over a hundred metabolites, compared group averages for all of them, and calculated pair-wise correlations between 14 metabolites. Importantly, for one hundred independent tests, one can expect to see five that satisfy $P < 0.05$ by chance alone, which could account for many of the 14 metabolites reported as age-related. We did not find a description of how this phenomenon was managed in the paper, which diminishes the confidence on the robustness of the reported findings.

Finally, we can benefit from the experience of replication in genetics. The flood of potential biomarkers in metabolomics resembles the era of candidate gene studies; numerous genetic variants were reported, but almost all eventually failed to replicate. Robust findings increased only after stringent requirements for large-scale genome-wide genotyping and independent replication were universally recognized. Thus, we need to incorporate replication in metabolomics studies of complex human conditions and biomarker discovery. We should also curb unfounded claims of complex epidemiological concepts, such as aging, that are known to be heterogeneous across time both at the individual and population level.

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