Comparative blood and urine metabolomics analysis of healthy elderly and young male Singaporeans

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Introduction

The relationship between Metabolomics, a powerful tool for unveiling metabolic details and screening important biomarkers of diseases, and Ageing could be a key contributor for the development of anti-ageing interventions but is yet to be fully understood.

Most of the reported human studies have been carried out on Caucasian cohorts or only investigated blood samples. Hence, a better understanding of the metabolic status of the rapidly increasing aging Asian population, especially in Singapore, epitome of a fast aging nation in the world, is important and urgently needed.

With this in mind, our study aimed to conduct multiple metabolome comparisons in different biofluid samples (blood and urine samples) from a Singaporean cohort to produce data that allows us to have a reference dataset to investigate drivers of ageing.

Methods

33 young and 33 elderly healthy chinese male adults underwent dietary recall, exercise screen and blood and urine samples collection for analysis.

Serum blood and Urine Analysis

Blood samples were analysed for whole blood hematology determinations and Nuclear Magnetic Resonance, NMR-based profiling for 112 lipoprotein subclasses, immunohematology (wr-CRP), and glucose fasting analysis using a Siemens ADVIA 1800 & ADVIA Chemistry XPT. Urine samples were also sent for metabolomics analysis.

Lipoproteins underwent quantitation and total detected metabolites and differential metabolites were submitted for pathway analysis in webtool MetaboAnalyst, which combines results from powerful pathway enrichment analysis with the pathway topology analysis.

Statistical Analysis

H NMR data were analyzed using SIMCA Multivariate Data Analysis. Principal component analysis (PCA) was performed to evaluate the sample clustering behavior and intragroup variation. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to maximize the metabolic differences between the sample groups.

H NMR spectral peaks were also integrated using an in-house algorithm and Mann–Whitney–Wilcoxon (MWW) tests, and adjusted false discovery rates were calculated.

Results

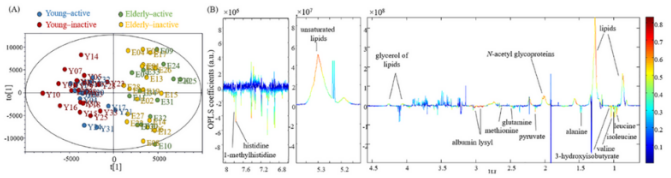

Figure 1. OPLS-DA score plots and loading plot of serum samples.

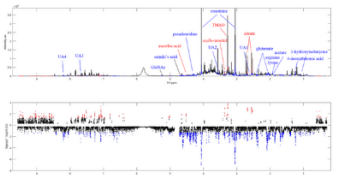

Figure 2. Urinary metabolome comparison between elderly and young subjects identified by 1H NMR

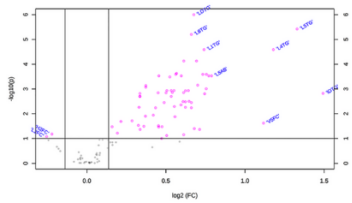

Figure 3. Volcano plot analysis of 112 lipoprotein subfractions between elderly and young.

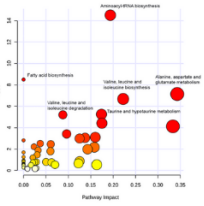

Figure 4. Pathwaytopology analysis in association with ageing

Increased serum metabolome levels of N-acetyl glycoproteins (NAG), lipids, glyceryl of lipids, and unsaturated lipids

Increased urinary metabolome levels of TMAO (trimethylamine N- oxide), scyllo-inositol, citrate, and ascorbic acid

Decreased serum metabolome levels of amino acids and organic acids (branched-chain amino acids (BCAA: isoleucine, valine, and leucine), histidine, alanine, 1-methylhistidine, glutamine, methionine, 3-hydroxyisobutyrate, pyruvate, and protein albumin lysyl)

Decreased urinary metabolome levels of creatinine, pseudouridine, 4-deoxythreonic acid, 3-hydroxyisobutyrate, arginine, lysine, acetate, glutamate, Sumiki's acid, N-acetylglucosamine (α/β GlcNAc), and unassigned metabolites UA1–UA4.

No observable significant difference between active and inactive subgroups in the elderly and young cohort, however, separation of the metabolome and lipoprotein profiles between elderly and young groups was evident.

Significant age-related metabolic signatures include increased serum levels of N-acetyl glycoproteins and several lipids versus decreased levels of albumin lysyl and essential amino acids and derivatives; increased urine levels of trimethylamine N-oxide, scyllo-inositol, citrate, and ascorbic acid versus decreased levels of several amino acids, acetate, etc.

Lipid signals were the most discriminatory with regards to aging. Among 112 lipoprotein subfractions in elderly, 65 were elevated while only 2 were lowered.

Significant alterations in age-related metabolites, notably amino acid and fatty acid metabolic pathways, point towards involvement in the aging process.

Discussion

Comparison of Serum Metabolome and Association of Amino Acid and Protein Metabolism with Aging

Several amino acid metabolic pathways were significantly different between the elderly and young, including proteinogenic amino acid metabolism (BCAA, alanine, aspartate, and glutamate) and nonproteinogenic amino acid metabolism (taurine and hypotaurine). This is significant to aging, and affect pathways in protein synthesis (1) and energy production and antioxidant production (such as carnosine and glutathione) (2, 3, 4, 5). N-acetyl glycoprotein, a clinical biomarker of systemic inflammation (6), was also raised in the elderly.

Comparison of Urine Metabolite Profiles between Elderly and Young Groups

Lower levels of proteinogenic amino acids were observed in elderly urine samples, including arginine, lysine, glutamate, and intermediate 3-hydroxyisobutyrate. This is associated with the decreased amino acid metabolism in elderly subjects

Biomarkers TMAO (associated with atherosclerosis, cardiovascular disease, and metabolic syndrome) (7), Scylloinositol, and ascorbic acid (possible association with supplementation) were higher in the elderly, while creatinine was lower in the elderly (indicating lower muscle index). Certain beneficial metabolites such as N-acetylglucosamine and acetic acid were also lower in the elderly. (8, 9, 10)

Lipid Metabolism Associated with Aging

Elevated levels of triglycerides were observed in all HDL, IDL, LDL, VLDL, and most of their subfractions in the elderly. In addition, in the elderly, increased cholesterol and phospholipids were also observed in IDL, LDL, VLDL, and especially in the more proatherogenic small dense LDL particles. Of the discriminatory subfractions between elderly and young subects, most belonged to the LDL and VLDL lipid class, suggesting increased endogenous LDL lipoprotein metabolism, which transfer triglycerides and cholesterol from the liver to peripheral tissues. (11) These observations imply that lipid and lipoprotein metabolic profiles could be sensitive indicators of aging.

Conclusion

Our study not only provides an important reference dataset reflecting the difference in component of blood and urine of Singaporean adults but also contributes to the growing evidence of the importance of amino acid and lipid metabolism in ageing.

Furthermore, diet choice and gut microbiome also factors in to the metabolome present hence further analysis on these two factors is required and will be carried out. A system interconnected network analysis with combination of metabolomics, metagenomics, and transcriptomics will enable a deeper understanding of these changes in the biological pathways with age and provide possible information that could assist in the design of antiaging intervention strategies or preventive measures in the Singaporean Chinese population.

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