Metabolic network analysis for understanding the biology of aging

Sudharshan Ravi*, Rudiyanto Gunawan*
*Institute of Chemical and Bioengineering, ETH Zurich, Switzerland
*Swiss Institute of Bioinformatics, Lausanne, Switzerland

Aging is a complex and multifactorial process that causes progressive functional decline and decreased ability of an organism to respond to stress. The complexity of the ageing process has motivated applying a systems-oriented approach through the creation and analysis of cellular networks. In this regard, recent human omics profiling efforts such as Genotype-Tissue Expression (GTEx), functional annotation of the mammalian genome (FANTOM), Human Protein Atlas and Human Metabolic Atlas projects, have generated large-scale human datasets, which would allow us to study human ageing process holistically. In this study, we focused on the relationship between ageing and metabolism. While this relationship has been well documented (Barzilai et al., 2012), most notably by the longevity effects of caloric or dietary restriction, a genome-wide analysis of human metabolism during aging has not yet been carried out. Here, we leveraged on transcriptomics data from the GTEx project and the curated genome-scale metabolic network model (Recon 2) (Thiele et al., 2013), to identify age-related metabolic alterations in human. We were also able to identify aging metabolic signatures in 11 human tissues. Our findings pointed to age-related alterations in pathways pertaining to cellular energy generation, branched-chain amino acid homeostasis, fatty acid metabolism and stress response.

The GTEx database consists of RNA-sequencing measurements of 56202 transcripts collected from 714 subjects whose ages ranges from 20 to 79 years old. Information on sample collection, quality control and normalization is available elsewhere (Mele et al., 2015). We employed a linear mixed effects model to determine the set of "aging genes", i.e. genes whose expression were altered with aging across different human tissues.

$$\mathbf{Y}_{ijk} = b_i + u_{ij}\,\mathbf{T}_j + x_{ik}\,(\mathbf{Sex})_k + z_i\,(\mathbf{Age})_k + \mathbf{g}_k + \mathbf{e}_{ijk}$$

where $\mathbf{Y}_{ijk}$ is the expression of gene i in tissue j belonging to the subject k, $u_{ij}\,\mathbf{T}_j$ and $x_{ik}\,(\mathbf{Sex})_k$ denote the fixed effects of tissue (T_j) and sex, respectively, and $\mathbf{g}_k$ is the random effect associated with each subject. To analyse tissue-specific gene expression changes, the linear mixed effect model was applied on a subset of the transcriptomic data belonging to that particular tissue, without the term $u_{ij}\,\mathbf{T}_j$. The number of differentially expressed genes with age (FDR<0.01), referred to below as aging genes, for 11 major tissues are tabulated in Table 1.

Table 1. Number of differentially expressed genes

Tissue	Number of Aging genes		
	Up	Down	Total
Overall	2968	4564	7532
Adipose	6505	816	7321
Artery	2229	2746	4975
Blood	126	3128	3254
Brain	28	7212	7240

Colon	4514	1271	5785
Esophagus	2442	1540	3982
Heart	17	435	452
Lung	1951	2446	4397
Muscle	2084	992	3076
Nerve	2996	611	3607
Skin	1342	643	1985

RECON 2 human genome-scale metabolic model consists of 2140 metabolic genes that control 4821 metabolic reactions. The reactions are classified into various subsystems based on their role and function. We mapped the aging genes to the human genome-scale metabolic network by identifying the metabolic reactions whose enzymes are among the aging genes (see Fig 1). Finally, we performed Fisher exact tests to determine the over-representation of different metabolic subsystems among the aging genes.

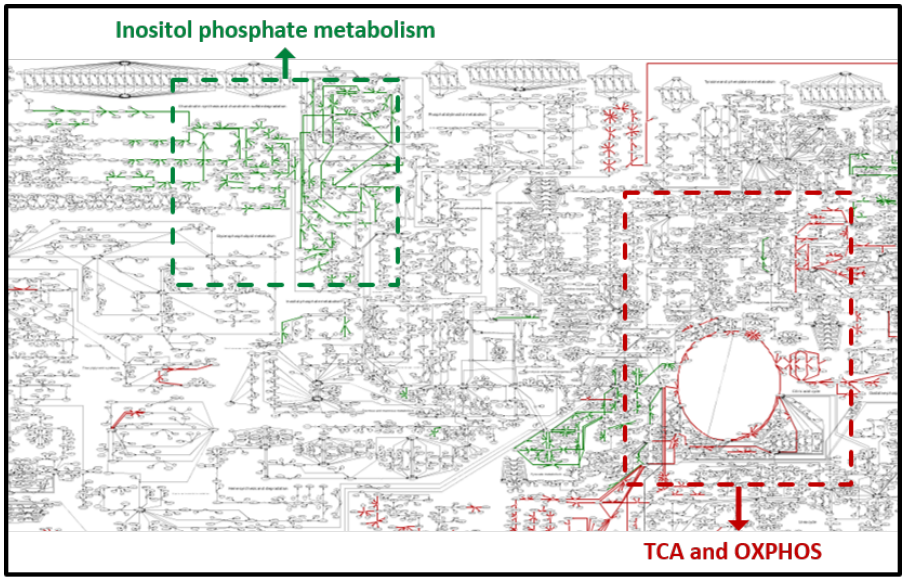

Fig. 1. Mapping the aging genes to the genome scale metabolic model allows us to look at over-represented subsystems by downregulated (highlighted in red) and upregulated (green) aging genes.

Our analysis pointed to significant age-related metabolic alterations of pathways pertaining to cellular energy generation. In particular, tricarboxylic acid (TCA) cycle and oxidative phosphorylation processes are overrepresented among the set of downregulated aging genes (see Fig 2A). These pathways are also over-represented among downregulated aging genes across multiple tissues, despite the varying number of overlapping aging genes and the number of aging genes among tissues. Metabolic pathways involved in the fatty acid oxidation are also downregulated across all tissues. Indeed, impaired fatty acid oxidation has previously been shown to be associated with mouse aging (Houtkooper et al., 2011). Meanwhile, inositol phosphate metabolism pathway is enriched among upregulated aging genes across different tissues (see Fig 2B). This pathway regulates cell proliferation and is also involved in the energy sensing pathway

PI3K/Akt. Our analysis also showed disruption of amino acid homeostasis (nucleotide interconversions) and branched chain amino acid metabolism.

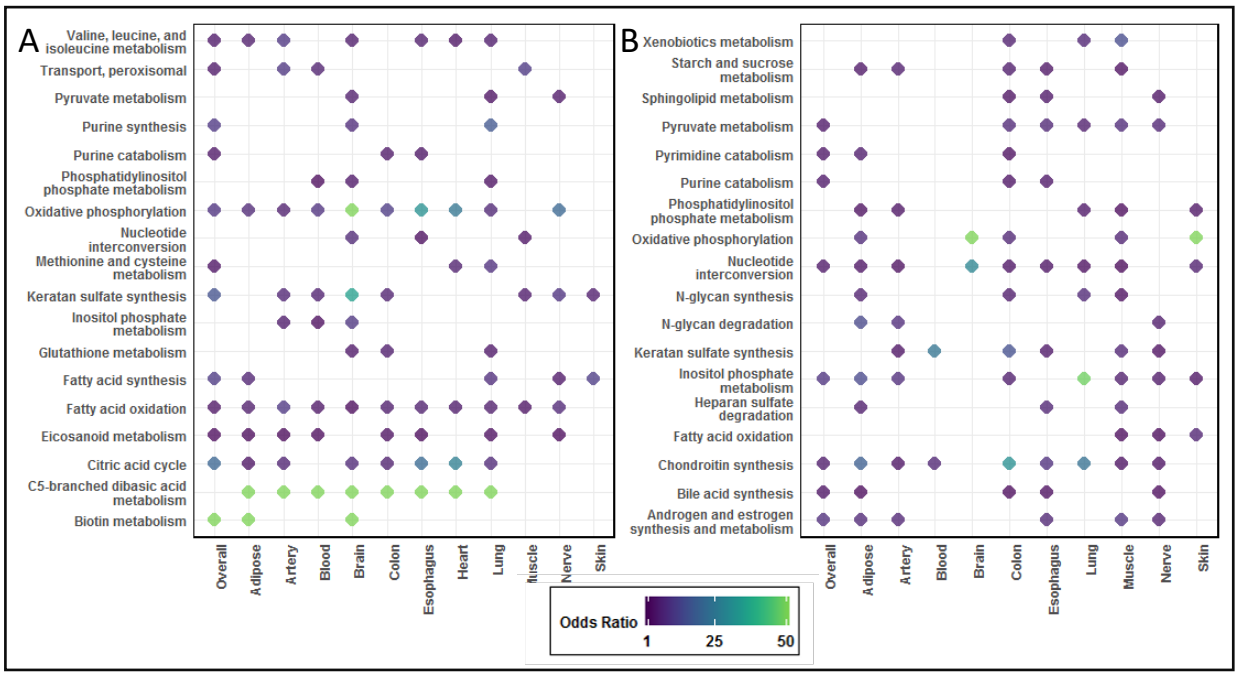

Fig. 2. Enriched subsystems across multiple tissues by (A) downregulated tissue specific aging genes and (B) upregulated tissue specific aging genes.

References

Lopez-Otin, C., Blasco, M.A., Partridge, L. and Kroemer, G. (2013). The Hallmarks of Aging. *Cell*, 153, 1194-1217.

Simon, C.J., Dong, X., Vijg, J., et al. (2015). Genetic evidence for common pathways in human age-related diseases. *Aging Cell*, 14, 809-817.

Barzilai, N., Huffman, D.M., Muzumdar, R.H. et al. (2012). The critical role of metabolic pathways in aging. *Diabetes*, 61, 1315-1322.

Thiele, I., Swainston, N., Fleming, R. and Palsson, B. (2013) A community-driven global reconstruction of human metabolism. *Nature Biotechnology*, 31, 419-425.

Mele, M., Ferreira, P., Reverter, F. and Guigo, R. (2015). The human transcriptome across tissues and individuals. *Science*, 348, 660-665.

Houtkooper, R.H., Argmann, C., Houten, S. and Auwerx, J. (2011). The metabolic footprint of aging in mice. *Scientific Reports*, 1, 134.