Transcriptomic studies in human ageing and longevity

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Chapter 9

Summary

The most prevalent diseases in the Western world have in common that age is the major risk factor. Life expectancy has increased dramatically in the last two centuries and will continue to rise. The healthy lifespan in western societies however, is usually not more than 75% of a lifetime. Many molecular changes occur as a function of chronological age in all tissues. Such changes could represent a marker of the biological ageing rate. The most valuable biomarkers of ageing rate would associate not only with chronological age and health status, but also with morbidity and mortality in prospective studies. To find these biomarkers we investigated chronological and biological ageing in long-lived families, collected in the Leiden Longevity Study (LLS). The LLS consists of nonagenarian sibling pairs, their middle-aged offspring and the partners thereof which act as controls. As compared to controls, the offspring has a lower prevalence of myocardial infarction, hypertension and type 2 diabetes and a lower mortality risk and are therefore regarded as healthy agers.

Transcriptomic studies for identification of human pathways and biomarkers of ageing have so far mainly focused on chronological ageing in several tissues (the largest studies investigated blood) and reported on enrichment for pathways like immune response, energy metabolism, mitochondrial processes and post-transcriptional regulation which may harbor potential biomarkers for chronological ageing. These studies have been reviewed in chapter 2 of this thesis. In this thesis we aimed for identification of relevant pathways and potential biomarker profiles that associate with chronological age in human populations and we tested which of these discriminate between healthy ageing members from long-lived families and normative ageing controls by studying the blood transcriptome using various approaches. Such pathways and biomarkers may harbor determinants of the biological ageing rate.

In chapter 3 we describe a genome-wide gene expression microarray study of 47,209 probes (representing 17,896 known genes) within 50 families of the LLS to screen for genes of which expression changes with age and associated with biological ageing. We identified 2,953 probes that associated with chronological age when comparing gene expression profiles from whole blood samples between 50 nonagenarians and 50 middle-aged controls, the latter representing the general population. From these, the expression of 360 probes was found to change differentially with age in offspring of the nonagenarians as compared to controls. In a RT-qPCR replication experiment utilizing 312 controls, 332 offspring and 79 nonagenarians, we confirmed a ‘‘chronological aging-signature’’ formed of 21 genes, of which reduced expression of *ASF1A* and *IL7R* marked familial

longevity already in middle-age. This indicates that expression changes of *ASF1A* and *IL7R*, which are involved in chromatin modeling and the immune system respectively, represent early features of familial longevity and healthy ageing.

The *IL7R* gene encodes the interleukin 7 receptor, which is important in immune response processes and critical for T cell development. Increased expression of IL7R and IL7 has been associated with prevalence of immune-related diseases. In chapter 4 we explore whether the expression of the *IL7R* gene and six interaction partners in blood associated with familial longevity, and whether the association is dependent on whole blood cell proportions and immune-related diseases including type 2 diabetes, COPD and rheumatoid arthritis. Investigating the expression of these seven genes in blood of the LLS using RT-qPCR resulted in a significant association of the network in a set-based test with chronological age ($p=4.6 \times 10^{-4}$). *IL7R* expression was found to be lower in the offspring as compared to the controls, which is in concordance with the observation that higher *IL7R* gene expression in blood is associated with higher prevalence of immune-related diseases (p-value=0.001). The association of lower IL7R gene expression with familial longevity was not explained by variation between the groups in blood cell counts and in the prevalence of the tested immune-related diseases. Paradoxically, *IL7R* gene expression decreases with age and higher *IL7R* gene expression levels associate with better survival in old and middle age. Taken together, the *IL7R* network reflected by gene expression levels in blood may mark the biological age and health status of elderly individuals, although the complexity of the mechanism still requires elucidation.

In addition to the explorative analysis, we also investigated candidate pathways for human longevity. The pathway that extensively has been implicated in lifespan extension but also development of disease in model organisms is mTOR signaling. This pathway has also been associated with diseases like diabetes and cancer in humans, but its impact on familial longevity or healthy ageing has not been determined yet. In chapter 5 we therefore investigated the gene expression level of the mTOR signaling pathway in blood within the LLS study. Forty genes were measured using RT-qPCR and as a set it showed significant association with chronological age when comparing mRNA levels of nonagenarians and middle-aged controls ($p=4.6 \times 10^{-7}$). Seven out of the 40 mTOR pathway genes had a significant differential expression of at least 5%. Of these, *RPTOR* showed a decreased expression in members of the long-lived families as compared to controls, already in middle-age, independent of prevalence of type 2

diabetes and cancer or variation in glucose levels. At the level of suggestive evidence this was also the case for *PRRL5*. Taken together, the mTOR pathway is not only involved in regulation of disease and lifespan in animal models, but also in humans. The gene expression in blood of *RPTOR* is a potential biomarker for biological ageing.

The mTOR pathway has been broadly studied in cell lines before, and cellular responses to stress in skin fibroblasts of offspring and controls from the LLS were shown to be associated with familial longevity. Therefore we questioned whether the mTOR pathway in this cellular model might reflect the metabolic characteristics of familial longevity. In chapter 6 we described this functional follow-up of the mTOR signaling results in blood, which was performed in skin fibroblasts from middle-aged LLS offspring with their partners as controls. Besides gene expression levels of all 40 mTOR signaling genes, expression level and phosphorylation of the most important proteins in the mTOR pathway were measured and analyzed, as well as total protein content and autophagy markers. However, only decreased total protein content was observed in offspring of long-lived individuals compared to controls. Besides not finding the same difference as was found in blood before, gene expression levels in fibroblasts did not reflect the corresponding protein levels in cultured fibroblasts or gene expression levels in whole blood in the same donors. Hence, the effect of mTOR signaling on human familial longevity may not be present in all tissues.

In chapter 7 we performed a meta-analysis combining multiple large-scale expression studies in blood to discover robust markers for chronological ageing in an integrative network-based approach. The combination of co-expression of genes and protein-protein interaction networks resulted in five robust networks significantly associated with normative ageing and included modules enriched for "*Translational elongation*", "*Cytolysis*" and "*DNA metabolic process*". An independent study in a Dutch cohort resulted in the validation of four out of five networks, indicating the robustness of our results. Whether these networks may be considered as markers for biological ageing as well has yet to be explored.

Striking is that five genes (*ABCE1*, *ASF1A*, *DCK, MPHOSPH10* and *ZZZ3*) of one of the robust networks are also among the 259 possible candidate markers for biological ageing as described in Chapter 3. An independent study also shows this network to be associated to survival at old age. As a relatively unknown group of genes it is important to further investigate this network in regards to function and relationship with health and ageing.

In this thesis we identified several pathways that change their gene expression as a function of chronological age. These may represent potential biomarkers in blood that associate with chronological age in human populations, including genes involved in translational elongation, regulation of transcription and lymphocyte development. We found indication that genes in these pathways may be relevant for ageing or could represent biomarkers for biological ageing, including expression levels of *ASF1A*, *IL7R*, *IL7* and *RPTOR*, which discriminate healthy agers (from longevity families) from normally ageing controls. Our explorative analysis resulted in 259 possible candidate markers for biological ageing of which 23 have been tested for replication in a larger part of the LLS. Of the yet untested genes, 99 were part of a large ageing-associated interaction network based on genes contributing to the mTOR pathway, IL7R network and the five robust co-expressed PPI modules. These 99, but also 4 other genes that formed a centrosome network separate from the large network, need to be validated in a larger part of the study population as possible markers for biological ageing.

A first step to investigate the biological relevance of the observed associations of the nutrient sensing mTOR pathway with familial longevity is initiated by the follow-up of these candidate genes in an intervention study called “Growing Old Together”. Offspring of nonagenarian siblings and their partners are subjected to an intervention of dietary restriction and physical exercise for 13 weeks. By examining the (candidate) biomarkers for the rate of ageing in blood, but also in muscle and fat, before and after the intervention, we aim to determine to what extent the controls change their molecular profile towards that of healthy agers and whether variation in mTOR expression associates to variation in response to the intervention. Such studies are ultimately aimed to stimulate middle aged individuals in the general population to improve their metabolic health and slow down their biological ageing rate and the gene expression profiles studied in this thesis are tested as monitoring tools for such improvements in health.