Chapter 1

General introduction

The life expectancy of the general western population has increased dramatically in the last 200 years (1) and is generally expected to continue this rise. Many people reach a high age, but at the same time on average 25% of the lifespan is spent in disability. For virtually all common diseases, age is the primary risk factor. The process of ageing is complex, generally considered as the consequence of accumulation of damage to macromolecules which occurs at a variable rate in different individuals, and affects organs in different ways, ultimately leading to physiological heterogeneity of elderly individuals, populations and patient groups. The scientific human ageing field focuses on identifying biomarkers that monitor the rate of ageing, markers of the biological age rather than just the chronological age of individuals. Either such markers are causally involved in ageing or can be used as tools to discover biological mechanisms that determine the ageing rate and the ability to age healthily and become long-lived.

Longevity determinants

The maximum and mean lifespan of animal models can be manipulated by intervening in a number of pathways (2-4) that indicate a role in lifespan regulation especially for nutrient sensing systems involving insulin/IGF (IIS) and target of rapamycin (TOR) signaling. These determinants have been investigated as candidate genes and pathways in human studies aimed at identifying determinants of human lifespan regulation. In particular, genetic association studies, in which gene frequencies are compared in highly aged cases and younger controls, provided positive associations for genes in the IIS pathway (5). For example, genetic variation in *AKT* and *FOXO3a* (6;7) has been shown to have an effect on human longevity. Until now however, the only locus widely confirmed to affect human mortality risk up to the highest ages is the *APOE* locus on chromosome 19 (8-10).

Biomarkers for human ageing

Studies for biomarkers of human ageing have mainly investigated the relation of physiological and molecular phenotypes with chronological age to identify changes in these phenotypes representative of normative ageing. Since many molecular changes occur as a function of chronological age, a more valuable biomarker of human ageing would also associate with morbidity and mortality in prospective studies (11) and thereby reflecting biological ageing. An example of such a biomarker is leukocyte telomere length (LTL) which associates with metabolic health and mortality in various human cohort studies including prospective analyses (12;13).

Nevertheless, this biomarker may not necessarily be a determinant of ageing since a causal role for telomere length in human normative ageing has not been established yet.

One strategy to find candidate biomarkers for human ageing is to compare molecular traits in normative and healthy ageing groups within the human population. The healthy ageing individuals can be selected from families in which the longevity trait is clustered. In the Longevity Genes Project such families have been collected by selection for centenarian single cases (14) whereas in the Leiden Longevity Study selection has been focused on nonagenarian siblings (15). As compared to normative controls, the middle-aged descendants of these centenarians and nonagenarians have a low prevalence of metabolic diseases such as insulin resistance, type 2 diabetes, hypertension and myocardial infarction (16) and lower mortality risk (15;17) and are therefore regarded as healthy agers.

Biomarker studies comparing the healthy ageing offspring of the long-lived to normative ageing controls have indicated that despite the absence of difference in body mass index and lifestyle between the groups, the offspring displays a healthier intrinsic metabolism reflected by serum lipid profiles (14;18), adiponectin levels (19) and markers of glucose metabolism (20). These measures of intrinsic metabolism are currently considered as candidate biomarkers for human ageing in middle age. For elderly populations however, the predictive power of these biomarkers for morbidity and mortality is less clear.

The transcriptome

Besides the serum parameters as candidate biomarkers for human ageing, an additional source for biomarkers may come from variation in gene expression. Genes and functionally coherent gene sets of which the gene expression changes with chronological age could set the profile for normative ageing, providing a first insight into the mechanisms of ageing processes and age-related disease (21). The last decade gene expression changes with age have regularly been investigated both in animal models and humans, using different designs, phenotypes and techniques. This work has been reviewed for studies until 2008 in chapter 2 of this thesis. In short, in different tissues in humans, the top most differentially expressed genes with chronological age have been enriched for biological terms like immune response, energy metabolism and mitochondrial processes. After 2008, explorative studies on age-related changes in the human transcriptome have fundamentally increased their power by increasing

sample sizes by mainly investigating RNA isolated from blood (21-23). While at the single gene level the mutual overlap of transcriptional changes with chronological age between different studies is limited, the enrichment is similar. The major consistent additional enrichment among the top list of genes of which gene expression changes with age since 2009 is for post-transcriptional regulation. Thus processes involved in immune response, energy metabolism, mitochondrial processes and post-transcriptional regulation may harbor potential biomarkers for the rate of normative ageing.

For practical reasons the largest human studies into gene expression changes with chronological age are naturally performed in blood. Smaller studies indicated that common gene sets are differentially expressed with ageing over different tissues (22;24). The similarities in age-affected pathways across tissues and species suggests that the analysis of the ageing transcriptome by functionally grouped gene sets in blood is a promising approach to discover biomarkers and determinants of biological ageing in humans.

Aim and outline of this thesis

The aim of this thesis was to identify in the human blood transcriptome, potential biomarker profiles that associate with chronological age and discriminate between 'healthy agers' from long-lived families and normative ageing controls. Such profiles may harbour determinants of the biological ageing rate.

The design for our study is based on the Leiden Longevity Study (LLS), a Dutch cohort in which families have been included on the basis of nonagenarian sibling pairs (15). The LLS consists of 420 Caucasian families with at least two long-lived siblings (n = 944), together with their middle-aged offspring (n = 1671) and the partners of this offspring (n = 744) which will act as controls in this study (Figure 2 of chapter 2 of this thesis). We have explored the blood transcriptome to recognize genes of which the expression marks chronological ageing (comparing groups of young and elderly individuals) and to identify which of those genes also mark familial longevity (comparing among middle-aged individuals groups of healthy and normative agers) (Chapter 3). Based on the top hits and most interesting findings from these explorative analyses we extended, within the Leiden Longevity Study, our analysis to a larger set of samples and genes belonging to the *IL7R* pathway (Chapter 4) and to the *MTOR* pathway (Chapter 5). The results of the latter study were followed by studies in primary fibroblasts cultures from donors of the LLS (Chapter 6). Finally to

improve and generate more robust and specific hypotheses for markers of normative ageing we performed an explorative meta-analysis in three large transcriptomic datasets using an integrative co-expressed network approach (Chapter 7).

References

(1) Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science 2002 May 10;296(5570):1029-31.

(2) Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009 July 16;460(7253):392-5.

(3) Kirkwood TL, Kapahi P, Shanley DP. Evolution, stress, and longevity. J Anat 2000 November;197 Pt 4:587-90.

(4) Lin K, Hsin H, Libina N, Kenyon C. Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat Genet 2001 June;28(2):139-45.

(5) Deelen J, Uh HW, Monajemi R, van HD, Thijssen PE, Bohringer S et al. Gene set analysis of GWAS data for human longevity highlights the relevance of the insulin/IGF-1 signaling and telomere maintenance pathways. Age (Dordr) 2011 November 24.

(6) Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, Nikolaus S et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc Natl Acad Sci U S A 2009 February 24;106(8):2700-5.

(7) Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K et al. FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci U S A 2008 September 16;105(37):13987-92.

(8) Deelen J, Beekman M, Uh HW, Helmer Q, Kuningas M, Christiansen L et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. Aging Cell 2011 August;10(4):686-98.

(9) Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. Nat Rev Genet 2006 June;7(6):436-48.

(10) Schachter F, Faure-Delanef L, Guenot F, Rouger H, Froguel P, Lesueur-Ginot L et al. Genetic associations with human longevity at the APOE and ACE loci. Nat Genet 1994 January;6(1):29-32.

(11) Sprott RL. Biomarkers of aging and disease: introduction and definitions. Exp Gerontol 2010 January;45(1):2-4.

(12) Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci 2011 April;66(4):421-9.

(13) Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003 February 1;361(9355):393-5.

(14) Barzilai N, Gabriely I, Gabriely M, Iankowitz N, Sorkin JD. Offspring of centenarians have a favorable lipid profile. J Am Geriatr Soc 2001 January;49(1):76-9.

(15) Schoenmaker M, de Craen AJ, de Meijer PH, Beekman M, Blauw GJ, Slagboom PE et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur J Hum Genet 2006 January;14(1):79-84.

(16) Westendorp RG, van HD, Rozing MP, Frolich M, Mooijaart SP, Blauw GJ et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. J Am Geriatr Soc 2009 September;57(9):1634-7.

(17) Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H et al. Life-long sustained mortality advantage of siblings of centenarians. Proc Natl Acad Sci U S A 2002 June 11;99(12):8442-7.

(18) Heijmans BT, Beekman M, Houwing-Duistermaat JJ, Cobain MR, Powell J, Blauw GJ et al. Lipoprotein particle profiles mark familial and sporadic human longevity. PLoS Med 2006 December;3(12):e495.

(19) Atzmon G, Pollin TI, Crandall J, Tanner K, Schechter CB, Scherer PE et al. Adiponectin levels and genotype: a potential regulator of life span in humans. J Gerontol A Biol Sci Med Sci 2008 May;63(5):447-53.

(20) Rozing MP, Westendorp RG, de Craen AJ, Frolich M, de Goeij MC, Heijmans BT et al. Favorable glucose tolerance and lower prevalence of metabolic syndrome in offspring without diabetes mellitus of nonagenarian siblings: the Leiden longevity study. J Am Geriatr Soc 2010 March;58(3):564-9.

(21) Harries LW, Hernandez D, Henley W, Wood AR, Holly AC, Bradley-Smith RM et al. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging Cell 2011 October;10(5):868-78.

(22) de Magalhaes JP, Curado J, Church GM. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 2009 April 1;25(7):875-81.

(23) Hong MG, Myers AJ, Magnusson PK, Prince JA. Transcriptome-wide assessment of human brain and lymphocyte senescence. PLoS One 2008;3(8):e3024.

(24) Zahn JM, Sonu R, Vogel H, Crane E, Mazan-Mamczarz K, Rabkin R et al. Transcriptional profiling of aging in human muscle reveals a common aging signature. PLoS Genet 2006 July;2(7):e115.