Chapter 8

Summary

One of the main problems in the Western world is the increase in years of life individuals spent in disability, which is mainly caused by the increased prevalence of diseases with increasing age. However, by identification of mechanisms driving healthy aging and protection from age-related diseases, we might be able to extend the disability-free life expectancy. In this thesis, we mainly focused on the genetic component of longevity, which has been estimated to explain ~25% of the variation in human lifespan.

In **Chapter 2** we have reviewed the different genetic studies that have thus far been performed to study healthy aging and longevity. In addition, we discussed several of the family-based and prospective studies that have been initiated to identify biomarkers of healthy aging. We propose that quantitative parameters (or profiles) must (1) show a change with chronological age, (2) discriminate individuals based on their biological age and/or genetic propensity for longevity, and associate with (3) known health parameters and (4) morbidity and/or mortality in prospective studies before consideration as biomarkers of healthy aging.

In **Chapter 3**, **4,** and **5** we tried to identify (novel) lifespan regulating loci using a genetic approach. Hence, we used two different methods, namely single single nucleotide polymorphism (SNP)-based genome-wide association study (GWAS) analysis (**Chapter 3** and **4**) and gene set analysis, which is able to determine the combined effect of SNPs on a trait (**Chapter 5**) In our first GWAS (**Chapter 3**), we identified one locus that associates with a decreased probability to survive to ages beyond 85 years, which is the previously implicated *TOMM40/APOE/APOC1* locus. In our extended GWAS, in individuals from all over Europe (**Chapter 4**), we confirmed the association of the *TOMM40/APOE/APOC1* locus with decreased survival to ages beyond 85 and 90 years (Table 8.1). In addition, we identified a novel locus that associates with an increased probability to survive to ages beyond 90 years (Table 8.1), which is located in an intergenic region on chromosome 5q33.3. As expected, prospective analysis showed that the minor allele of the lead SNP at the *TOMM40/APOE/APOC1* locus (rs4420638) associates with increased mortality, while the minor allele of the lead SNP at the chromosome 5q33.3 locus (rs2149954) associates with decreased mortality (Table 8.1). In **Chapter 3** we showed that the association at the *TOMM40/APOE/APOC1* locus is caused by the ApoE ε4 defining SNP rs429358, which has previously been associated with an increased risk of cardiovascular disease and Alzheimer's disease and unfavorable levels of several metabolic phenotypes, such as total/high-density lipoprotein/low-density lipoprotein cholesterol and C-reactive protein. We additionally show an effect of ApoE ε4 on insulin-like growth factor 1 (IGF-1) signaling in women. The locus on chromosome 5q33.3, on the other hand, has previously been associated with lower systolic and diastolic blood pressure in middle age. However, we showed that the association of the locus with decreased mortality above 75 years is not explained by its relation with blood pressure and, most likely, also involves other traits (**Chapter**

4). In the gene set analysis (**Chapter 5**) we showed that genetic variation in genes involved in the insulin/IGF-1 signaling (IIS) and telomere maintenance (TM) pathways is associated with human longevity. Hence, gene set analysis may be used, in addition to GWAS, to study the combined effect of genetic variation in (known) genes and pathways on longevity.

Since our genetic studies identified a limited number of longevity loci, we additionally examined whether leukocyte telomere length (LTL) could be used as a biomarker of healthy aging in genomic studies of large cohorts of middle-aged individuals (**Chapter 6**). We showed that LTL meets three of the four criteria for a biomarker of healthy aging in the Leiden Longevity Study (LLS), i.e., LTL changes with chronological age and is associated with prospective mortality and immune-related parameters (Table 8.2). However, this is still insufficient to use LTL as a standardized phenotype for genetic studies of healthy aging and longevity. Thus, we need to search for parameters that meet all four proposed criteria for biomarkers of healthy aging.

When novel longevity loci, such as the chromosome 5q33.3 locus, have been identified, functional characterization needs to be performed to determine the mechanism underlying the association with healthy aging and longevity. This process consist of several steps, namely (1) genotypic fine-mapping, (2) phenotypic fine-mapping, (3) expression/epigenetic quantitative trait locus analysis, and (4) functional assays in model systems (animals/cell models). Functional characterization of the chromosome 5q33.3 locus showed that the locus encompasses a

Table 8.1 Longevity-associated genetic variants identified through genome-wide association studies.

						Survival ≥ 90 years		Mortality		
Locus	Lead SNP	Chr	Position (bp)	Causal SNP(s)	Candidate / closest gene	OR	*P*	HR	*P*	Previously reported associations
5q33.3	rs2149954	2	157,753,180	Unknown	*EBF1*	1.10	1.74×10^{-8}	0.95	0.003	Blood pressure
19q13.32	rs4420638	19	50,114,786	rs429358 (ApoE ε4)	*APOE*	0.72	3.40×10^{-36}	1.07	0.019	Cardiovascular disease, Alzheimer's disease, metabolic phenotypes

Chr, chromosome according to NCBI Build 36; *Position (bp)*, position of the lead SNP according to NCBI Build 36; *OR*, odds ratio; *HR*, hazard ratio.

Table 8.2 Association of leukocyte telomere length with chronological age, prospective mortality, familial longevity and immune-related parameters in the Leiden Longevity Study (LLS) offspring and partners and LLS nonagenarians.

	LLS offspring + partners		LLS nonagenarians	
	β / HR	*P*	β / HR	*P*
Age (years)	-0.040	0.002	-0.044	0.003
Prospective mortality	0.75	0.001	0.92	0.028
Familial longevity	0.006	0.932	NA	NA
IGF-1/IGFBP3 (molar ratio)*	0.052	1.19×10^{-5}	-0.004	0.800
CRP (mg/L)**	-0.007	0.802	0.008	0.821
IL-6 (pg/ml)	0.099	0.010	NA	NA
CMV infection	-0.168	0.005	-0.305	1.57×10^{-4}
Lymphocyte count (%)	-0.015	1.84×10^{-4}	-0.013	0.011
Neutrophil count (%)	0.014	1.51×10^{-4}	0.013	0.006
Monocyte count (%)	-0.020	0.383	-0.016	0.502
Eosinophil count (%)	-0.007	0.727	-0.038	0.123
Basophil count (%)	0.353	3.63×10^{-5}	-0.263	0.019

HR, hazard ratio; *IGF-1*, insulin-like growth factor 1; *IGFBP3*, insulin-like growth factor binding protein 3; *CRP*, C-reactive protein; *IL-6*, interleukin 6; *CMV*, cytomegalovirus. The LTL outcome used for this analysis represents the number of 1 kb telomeric base pair units. *The outcome represents the effect of a 0.01 increase in the parameter. **Natural log transformed parameter was used in the analysis.

~22.3 kb region containing several functional elements, such as DNase I hypersensitivity sites, as well as a long intergenic noncoding RNA, which is conserved in primates. However, we have thus far not identified diseases or traits, additional to blood pressure, associating with the locus. In addition, it is still unclear on which gene(s) and in which tissue(s) the locus exerts its effects.

To identify novel longevity loci, future studies should use stricter criteria for selection of individuals for genetic research to reduce the phenotypic heterogeneity. In addition, genetic studies may use biomarkers of healthy aging, preferable combined into one multimarker score, as a standardized phenotype for genetic studies. Identification of such biomarkers may profit from combining family-based studies, like the LLS, and population-based prospective studies with long follow-up times, since this will allow testing of all four proposed criteria for biomarkers of healthy aging. Furthermore, research into human lifespan may benefit from novel technologies and methodologies, such as next-generation sequencing, multigenerational linkage analysis, omics-based measurements, and multimarker prediction scores. Subsequent integration of the created data over different species, or in a system biology approach, may provide insight into the complex mechanisms underlying lifespan regulation.