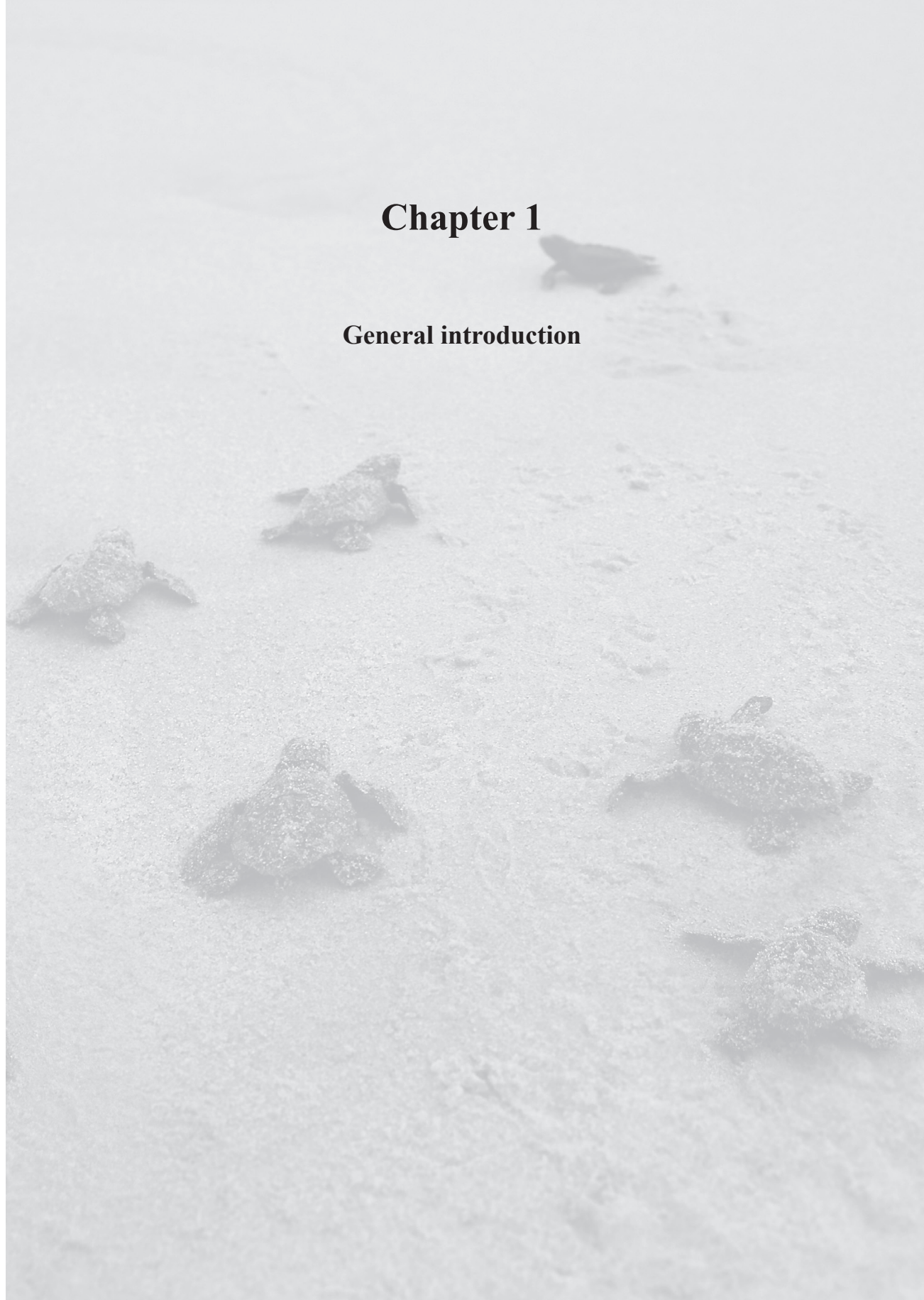
Chapter 1

General introduction



Worldwide, life expectancy has shown a remarkable linear increase over the last two centuries [1]. However, the number of years of life spent in disability also increases and individuals from the European Union born in 2009 are expected to spend on average 25% (women) or 20% (men) of their life in poor health, i.e., experience limited or severe long-term limitation (> 6 months) in usual activity caused by ill-health (http://www.healthy-life-years.eu/) [2]. This stresses the importance of efforts aimed at increasing the disability-free life expectancy. The majority of disabilities are caused by diseases, such as cancer, cardiovascular disease, hypertension, osteoarthritis, and type 2 diabetes, for which chronological age is the main risk factor. Interestingly, part of the individuals that survive to exceptionally old ages do not display excessive levels of disability [3,4], indicating that reaching a high age does not necessarily result in an increase in age-related disability.

Use of family-based cohorts to study healthy aging and longevity

It is expected that the number of years spent in disability could be reduced by avoiding age-related diseases [5]. Remarkably, long-lived families indeed display a low prevalence of age-related diseases from middle age onwards [6-10]. In addition, they show beneficial or "youthful" profiles for numerous cognitive, metabolic, and immune-related parameters. Examples of these features are the low prevalence of cytomegalovirus infections, low free triiodothyronine and triglyceride serum levels, and preservation of insulin sensitivity in middle age [7,11-16]. Thus, by studying long-lived families (Table 1.1), one might be able to identify mechanisms driving healthy aging and protection from age-related diseases in middle and old age. Ultimately, this knowledge may be used to extend the disability-free life expectancy in the population.

One strategy to identify the mechanisms underlying lifespan regulation is by applying genetic approaches. The genetic component of longevity, as estimated from twin and family-based studies, is ~25% (Table 1.2) and the genetic contribution increases with age [17,18]. However, there is large heterogeneity in the genetic component estimates between studies, which could be caused by geographical or methodological differences [19]. The genetic component is most prominent in long-lived families [20,21], which makes them highly suitable for genomic approaches.

In addition to the genetic approach, research into biological and physiological phenotypes accompanying a long life may illuminate mechanisms of healthy aging. To this end, long-lived families are being studied for quantitative parameters or profiles that mark chronological and/or biological age, i.e., the age based on the molecular and psychological functioning of the individual, which could subsequently be investigated in large cohorts of middle-aged individuals. Thus, identifying the genetic component and/or biomarkers of longevity may contribute to the disclosure of mechanisms driving healthy aging and longevity.

Table 1.1 Overview of family-based longevity studies.

	Long-lived individuals		Offspring	Controls		
Study	***n***	**Type**	***n***	***n***	**Type**	**Reference**
Ashkenazi Jews	365	Centenarians	593	356	Spouses of offspring + population controls	22
European Challenge for Healthy Ageing	257	Centenarians	276	204	Cousins of offspring without centenarian parent	23
GEnetics of Healthy Ageing*	4,498	Nonagenarian siblings	~700	2,249	Population controls	24
Leiden Longevity Study	944	Nonagenarian siblings	1,671	744	Spouses of offspring	21
Long Life Family Study	1,373	Nonagenarian siblings	2,317	582	Spouses of offspring	7
New England Centenarian Study**	>1,800	Centenarians	>600	437	Population controls	25

*Offspring is recruited in the MARK-AGE project, **Recruitment still ongoing.

Table 1.2 Overview of studies that examined the genetic component of longevity or lifespan.

			Total		Men		Women		
Study	**Country**	**Type**	***n***	h^2	***n***	h^2	***n***	h^2	**Reference**
GenomEUtwin	Denmark / Finland / Italy / Sweden	Twins	9,334		4,598	0.120	4,736	0.260	26
Danish Twin Registry	Denmark	Twins	5,744		2,816	0.260	2,928	0.230	27
Swedish Twin Registry	Sweden	Twins	1,250		164	0.010	194	0.150	28
Utah Population Database	United States	Families	78,994	0.147					29
European royal and noble families	Europe	Families	12,150		8,409	0.180	3,741	0.200	17
MICROS study	Italy	Families	8,277	0.150	4,299	0.160	3,978	0.180	19
Genealogia Sursilliana CD-2000	Finland	Families	2,614		1,226	0.175	1,388	0.167	30
Old Order Amish	United States	Families	1,655	0.250					31
Valserine Valley XVIII-XXth Centuries	France	Families	1,102	0.270	586		516		32

h^2, genetic component estimate (heritability or comparable statistic).

Genetic research of aging and longevity in animal models

The first studies into the genetics of lifespan regulation were performed in animal models, such as yeast, worms, flies, and mice. In contrast to human longevity studies, which are mainly observational, animal-based studies benefit from genetic manipulation (mutagenesis) via RNA interference, knock-out or overexpression of single genes. Using these approaches, many genes have been identified that extend lifespan in these models (GenAge; http://genomics.senescence.info/genes/) [33]. The most interesting conserved pathways identified using animal models are the growth hormone (GH)/insulin/insulin-like growth factor 1 (IGF-1) signaling and mammalian target of rapamycin signaling pathways [34]. The limitation of the animal-based longevity studies in lower species, such as worms, is that they mainly focus on lifespan as an outcome and that the parameters that reflect the physiology and pathology of aging are not well defined or highly difficult to compare with their human counterparts. Nonetheless, these studies have been crucial for the identification of lifespan regulating pathways that also contribute to human longevity.

Application of GWAS for identification of novel human longevity loci

Most genetic research on human longevity has been focused on lifespan regulating loci involved in GH/insulin/IGF-1 signaling [35]. Although many of the GH/insulin/IGF-1 signaling genes have been investigated (see http://genomics.senescence.info/longevity/ [36] for an overview), the only gene associated with human longevity in multiple independent studies is *FOXO3A* [37-39]. *FOXO3A* encodes the protein forkhead box O3, which acts as a transcription factor for many different genes involved in, e.g., apoptosis and oxidative stress [40]. In addition, a study by van Heemst and colleagues showed that a composite pathway score based on 6 genetic variants in GH/insulin/IGF-1 signaling genes is associated with mortality in women, which further highlights the role of this pathway in lifespan regulation [41]. The other candidate gene that has consistently been associated with human longevity in multiple independent studies is *APOE* [35,42]. *APOE* encodes the protein apolipoprotein E (ApoE), which seems to be involved in, e.g., lipoprotein metabolism, cognitive function, and immune regulation [43]. The ApoE protein has three isoforms (ApoE ε2, ApoE ε3, and ApoE ε4) defined by two single nucleotide polymorphisms (SNPs), rs7412 (Arg136Cys; ε2) and rs429358 (Cys112Arg; ε4). Interestingly, ApoE ε4 has been associated with a decreased probability to become long-lived, while ApoE ε2 has an opposite effect. However, since the effect of ApoE ε4 seems to be most prominent, *APOE* is generally considered a "frailty gene" [44]. Thus, although candidate gene studies have shown to be useful, the number of human longevity genes identified by these studies is limited.

Instead of studying the genome using a hypothesis-based approach, hypothesis-free approaches could be performed. An example of such an approach is the genome-

wide association study (GWAS), aimed at identifying common genetic variants with, usually, small effects. In a GWAS, 300,000-2,500,000 SNPs are assessed for association with the trait of interest. This approach has successfully been applied to many diseases and traits (National Human Genome Research Institute GWAS Catalog; http://www.genome.gov/gwastudies/) [45]. In GWAS for longevity, genotype frequencies are compared between long-lived cases and shorter-lived or young controls. The genome of long-lived individuals is assumed to be characterized by a decreased prevalence of disease-promoting variants of considerable effect and an increased prevalence of variants promoting healthy aging. Since longevity is assumed to be determined by many genes with small effects, GWAS is expected to be a successful method to identify novel human longevity loci.

Genomic research might benefit from biomarker research

The number of long-lived individuals that can currently be included in genomic studies is limited (~30,000 individuals). Hence, it is almost impossible to reach a sufficient sample size required to identify genetic variants with relatively small effects, such as those identified for more common traits, like height and lipid levels, with sample sizes > 100,000 individuals. To overcome this problem, one might try to identify (combinations of) phenotypes that could be used as biomarkers of healthy aging in genomic studies of large cohorts of middle-aged individuals. We propose that a biomarker of healthy aging should; (1) show a change with chronological age, at least above 40 years, (2) discriminate individuals with a "youthful" or old level relative to their age category in the general population, (3) associate with known health parameters, and (4) associate with future morbidity and/or mortality in prospective studies (**Chapter 2**).

Aim and outline of the thesis

The drivers of human longevity may provide insight in the mechanisms that result in delay or avoidance of age-related diseases. Since knowledge of such mechanisms may contribute to the extension of disability-free lifespan, the aim of this thesis was to identify novel lifespan regulating loci that influence human longevity and population mortality. We performed our research in various cohorts of elderly individuals, including the family-based Leiden Longevity Study (LLS) and GEnetics of Healthy Ageing project (Table 1.1), the population-based Rotterdam Study, which includes individuals above 55 years that were followed-up for > 20 years, and the prospective Leiden 85-plus study and PROspective Study of Pravastatin in the Elderly at Risk, in which the association of a genetic variant with mortality can be tested.

To identify genetic drivers of human longevity by GWAS, we first compared unrelated nonagenarians from the LLS (Table 1.1) with young controls from the Rotterdam Study. The loci that showed suggestive evidence for association with survival ≥ 90 years were tested for replication in the Rotterdam Study, Leiden 85-plus study, and Danish 1905 cohort. Subsequently,

we performed a combined analysis of the discovery and replication cohorts (4,149 cases and 7,582 controls) (**Chapter 3**).

Due to the complexity of the longevity phenotype and the relatively small sample size, the LLS longevity GWAS turned out to have insufficient power to detect significant effects besides the well-established *TOMM40/APOE/APOC1* locus (**Chapter 3**). We therefore carried out an extended GWAS, in which we studied the genetics of long-lived cases (≥ 85 years) and younger controls (< 65 years of age) from all over Europe. The loci that showed suggestive evidence for association with survival ≥ 85 and/or ≥ 90 years were taken forward for replication in 6 additional cohorts and we performed a combined analysis of the discovery and replication cohorts (20,789 cases and 77,277 controls) (**Chapter 4**).

Instead of analyzing single SNPs, as was done in the LLS and EU longevity GWAS (**Chapter 3** and **4**), the combined effect of a SNP set, grouped per pathway or gene region, can be tested for association with longevity. The advantage of these tests is that they are very suitable for studies of polygenic complex traits with limited power for GWAS analysis, such as longevity [46], due to the low penalty for multiple testing as compared to single SNP analysis. Two candidate pathways for human longevity are the insulin/IGF-1 signaling (IIS) pathway and the telomere maintenance (TM) pathway. The IIS pathway is involved in the adaptation of the organism to its (changing) environment [47], while the TM pathway regulates telomere integrity [48,49]. Genetic variation in genes that play a role in IIS and TM has previously been associated with human longevity [37,39,41,50]. To determine if the combined effect of IIS and TM pathway SNPs is associated with human longevity, we performed gene set analysis with gene sets based on these pathways using the LLS longevity GWAS dataset (**Chapter 5**).

Since our genetic approaches delivered a limited number of longevity loci and pathways, we also performed a study on leukocyte telomere length (LTL), a potential biomarker of healthy aging that could be used for genomic studies in large cohorts of middle-aged individuals. Previous studies have shown that LTL is associated with multiple diseases and increased prospective mortality [51]. In addition, a study in an Ashkenazi Jewish population (Table 1.1) showed that offspring of centenarians have a longer mean LTL as compared to controls from the general population [50], indicating that mechanisms regulating LTL might also be involved in human lifespan regulation. Hence, to test the proposed criteria for biomarkers of healthy aging, we investigated LTL for association with chronological age, familial longevity, known health parameters, and prospective mortality in long-lived families from the LLS (**Chapter 6**). In addition, we performed a look-up of the LTL-associated genetic variants in our EU longevity GWAS results described in **Chapter 4** to determine the association with survival to ages beyond 90 years.

References

1. Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. *Science* 2002; **296**: 1029-31.
2. Jagger C, Gillies C, Moscone F, Cambois E, van Oyen H, Nusselder W, *et al*. Inequalities in healthy life years in the 25 countries of the European Union in 2005: a cross-national meta-regression analysis. *Lancet* 2008; **372**: 2124-31.
3. Christensen K, McGue M, Petersen I, Jeune B, Vaupel JW. Exceptional longevity does not result in excessive levels of disability. *Proc Natl Acad Sci U S A* 2008; **105**: 13274-9.
4. Terry DF, Sebastiani P, Andersen SL, Perls TT. Disentangling the roles of disability and morbidity in survival to exceptional old age. *Arch Intern Med* 2008; **168**: 277-83.
5. Jagger C, Matthews R, Matthews F, Robinson T, Robine JM, Brayne C. The burden of diseases on disability-free life expectancy in later life. *J Gerontol A Biol Sci Med Sci* 2007; **62**: 408-14.
6. Atzmon G, Schechter C, Greiner W, Davidson D, Rennert G, Barzilai N. Clinical phenotype of families with longevity. *J Am Geriatr Soc* 2004; **52**: 274-7.
7. Newman AB, Glynn NW, Taylor CA, Sebastiani P, Perls TT, Mayeux R, *et al*. Health and function of participants in the Long Life Family Study: A comparison with other cohorts. *Aging (Albany NY)* 2011; **3**: 63-76.
8. Bos SD, Beekman M, Maier AB, Karsdal MA, Kwok WY, Bay-Jensen AC, *et al*. Metabolic health in families enriched for longevity is associated with low prevalence of hand osteoarthritis and influences OA biomarker profiles. *Ann Rheum Dis* 2013; **72**: 1669-74.
9. Terry DF, Wilcox MA, McCormick MA, Pennington JY, Schoenhofen EA, Andersen SL, *et al*. Lower all-cause, cardiovascular, and cancer mortality in centenarians' offspring. *J Am Geriatr Soc* 2004; **52**: 2074-6.
10. Westendorp RG, van Heemst D, 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; **57**: 1634-7.
11. Stijntjes M, de Craen AJ, van Heemst D, Meskers CG, van Buchem MA, Westendorp RG, *et al*. Familial longevity is marked by better cognitive performance at middle age: the leiden longevity study. *PLoS One* 2013; **8**: e57962.
12. Barral S, Cosentino S, Costa R, Matteini A, Christensen K, Andersen SL, *et al*. Cognitive function in families with exceptional survival. *Neurobiol Aging* 2012; **33**: 619-7.
13. Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R, *et al*. Unique lipoprotein phenotype and genotype associated with exceptional longevity. *JAMA* 2003; **290**: 2030-40.
14. Derhovanessian E, Maier AB, Beck R, Jahn G, Hahnel K, Slagboom PE, *et al*. Hallmark features of immunosenescence are absent in familial longevity. *J Immunol* 2010; **185**: 4618-24.
15. Slagboom PE, Beekman M, Passtoors WM, Deelen J, Vaarhorst AA, Boer JM, *et al*. Genomics of human longevity. *Philos Trans R Soc Lond B Biol Sci* 2011; **366**: 35-42.
16. Wijsman CA, Rozing MP, Streefland TC, le Cessie S, Mooijaart SP, Slagboom PE, *et al*. Familial longevity is marked by enhanced insulin sensitivity. *Aging Cell* 2011; **10**: 114-21.
17. Gavrilova NS, Gavrilov LA, Evdokushkina GN, Semyonova VG, Gavrilova AL, Evdokushkina NN, *et al*. Evolution, mutations, and human longevity: European royal and noble families. *Hum Biol* 1998; **70**: 799-804.
18. Hjelmborg JV, Iachine I, Skytthe A, Vaupel JW, McGue M, Koskenvuo M, *et al*. Genetic influence on human lifespan and longevity. *Hum Genet* 2006; **119**: 312-21.
19. Gogele M, Pattaro C, Fuchsberger C, Minelli C, Pramstaller PP, Wjst M. Heritability analysis of life span in a semi-isolated population followed across four centuries reveals the presence of pleiotropy between life span and reproduction. *J Gerontol A Biol Sci Med Sci* 2011; **66**: 26-37.

20. Perls T, Shea-Drinkwater M, Bowen-Flynn J, Ridge SB, Kang S, Joyce E, *et al*. Exceptional familial clustering for extreme longevity in humans. *J Am Geriatr Soc* 2000; **48**: 1483-5.
21. 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; **14**: 79-84.
22. Lai JY, Atzmon G, Melamed ML, Hostetter TH, Crandall JP, Barzilai N, *et al*. Family history of exceptional longevity is associated with lower serum uric acid levels in Ashkenazi Jews. *J Am Geriatr Soc* 2012; **60**: 745-50.
23. De Rango F, Dato S, Bellizzi D, Rose G, Marzi E, Cavallone L, *et al*. A novel sampling design to explore gene-longevity associations: the ECHA study. *Eur J Hum Genet* 2008; **16**: 236-42.
24. Skytthe A, Valensin S, Jeune B, Cevenini E, Balard F, Beekman M, *et al*. Design, recruitment, logistics, and data management of the GEHA (Genetics of Healthy Ageing) project. *Exp Gerontol* 2011; **46**: 934-45.
25. Perls TT, Bochen K, Freeman M, Alpert L, Silver MH. Validity of reported age and centenarian prevalence in New England. *Age Ageing* 1999; **28**: 193-7.
26. Skytthe A, Pedersen NL, Kaprio J, Stazi MA, Hjelmborg JV, Iachine I, *et al*. Longevity studies in GenomEUtwin. *Twin Res* 2003; **6**: 448-54.
27. Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. *Hum Genet* 1996; **97**: 319-23.
28. Ljungquist B, Berg S, Lanke J, McClearn GE, Pedersen NL. The effect of genetic factors for longevity: a comparison of identical and fraternal twins in the Swedish Twin Registry. *J Gerontol A Biol Sci Med Sci* 1998; **53**: M441-M446.
29. Kerber RA, O'Brien E, Smith KR, Cawthon RM. Familial excess longevity in Utah genealogies. *J Gerontol A Biol Sci Med Sci* 2001; **56**: B130-B139.
30. Pettay JE, Kruuk LE, Jokela J, Lummaa V. Heritability and genetic constraints of life-history trait evolution in preindustrial humans. *Proc Natl Acad Sci U S A* 2005; **102**: 2838-43.
31. Mitchell BD, Hsueh WC, King TM, Pollin TI, Sorkin J, Agarwala R, *et al*. Heritability of life span in the Old Order Amish. *Am J Med Genet* 2001; **102**: 346-52.
32. Cournil A, Legay JM, Schachter F. Evidence of sex-linked effects on the inheritance of human longevity: a population-based study in the Valserine valley (French Jura), 18-20th centuries. *Proc Biol Sci* 2000; **267**: 1021-5.
33. de Magalhaes JP, Budovsky A, Lehmann G, Costa J, Li Y, Fraifeld V, *et al*. The Human Ageing Genomic Resources: online databases and tools for biogerontologists. *Aging Cell* 2009; **8**: 65-72.
34. Fontana L, Partridge L, Longo VD. Extending healthy life span--from yeast to humans. *Science* 2010; **328**: 321-6.
35. Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. *Nat Rev Genet* 2006; **7**: 436-48.
36. Tacutu R, Craig T, Budovsky A, Wuttke D, Lehmann G, Taranukha D, *et al*. Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing. *Nucleic Acids Res* 2013; **41**: D1027-D1033.
37. 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; **106**: 2700-5.
38. Kuningas M, Magi R, Westendorp RG, Slagboom PE, Remm M, van Heemst D. Haplotypes in the human Foxo1a and Foxo3a genes; impact on disease and mortality at old age. *Eur J Hum Genet* 2007; **15**: 294-301.
39. 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; **105**: 13987-92.

40. van der Horst A, Burgering BM. Stressing the role of FoxO proteins in lifespan and disease. *Nat Rev Mol Cell Biol* 2007; **8**: 440-50.
41. van Heemst D, Beekman M, Mooijaart SP, Heijmans BT, Brandt BW, Zwaan BJ, *et al*. Reduced insulin/IGF-1 signalling and human longevity. *Aging Cell* 2005; **4**: 79-85.
42. 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; **6**: 29-32.
43. Mahley RW, Rall SC, Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000; **1**: 507-37.
44. Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW. Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a "frailty gene," not a "longevity gene". *Genet Epidemiol* 2000; **19**: 202-10.
45. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, *et al*. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A* 2009; **106**: 9362-7.
46. Finch CE, Tanzi RE. Genetics of aging. *Science* 1997; **278**: 407-11.
47. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; **299**: 1346-51.
48. Collins K, Mitchell JR. Telomerase in the human organism. *Oncogene* 2002; **21**: 564-79.
49. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005; **19**: 2100-10.
50. Atzmon G, Cho M, Cawthon RM, Budagov T, Katz M, Yang X, *et al*. Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. *Proc Natl Acad Sci U S A* 2010; **107 Suppl 1**: 1710-7.
51. Sanders JL, Newman AB. Telomere Length in Epidemiology: A Biomarker of Aging, Age-Related Disease, Both, or Neither? *Epidemiol Rev* 2014; *In press*.