Genetic determinants of healthy longevity

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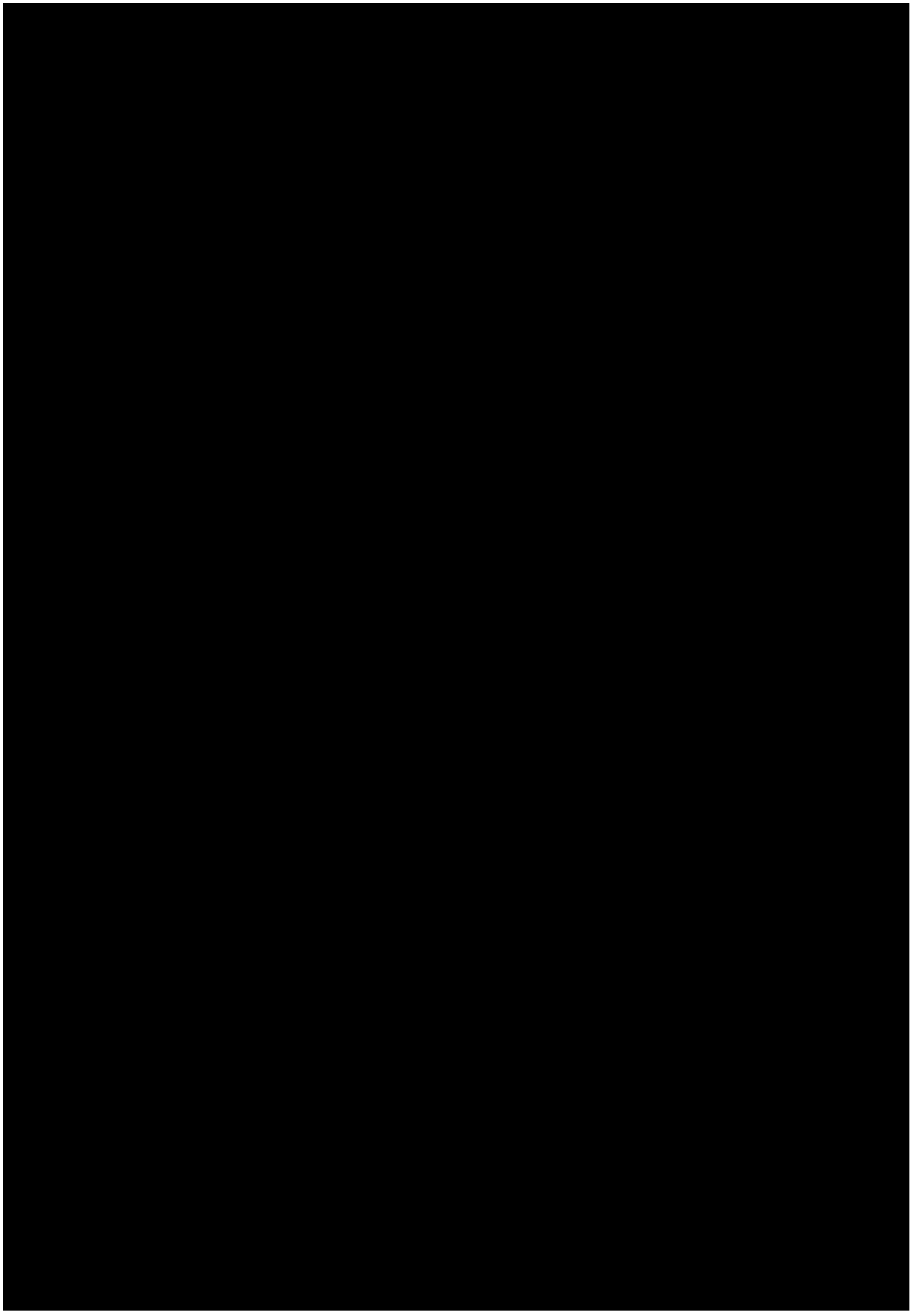
CHAPTER 1

General introduction



HEALTHY LONGEVITY IN HUMANS IS GENETICALLY DETERMINED

The length and quality of human life is under genetic control. This is illustrated by the fact that siblings of centenarians have a significantly higher chance of becoming centenarians themselves (Perls et al., 2002). Interestingly, the survival benefits of family-members of long lived subjects are lifelong and exist up to the highest age category (Hjelmborg et al., 2006; Perls et al., 2002). Furthermore, offspring of long lived sibling pairs have a lower mortality already at middle age, whereas their spouses, with whom they have shared decades of common environment, do not have this survival benefit (Schoenmaker et al., 2006). To date, little is known about genetic factors influencing length and quality of human life.

THE HUMAN GENOME

The human genome includes approximately thirty thousand genes. The variation in these genes results from thousands of years of natural selection. In this 'experiment by nature' consecutive generations have optimized and shaped the population genome through Darwinian fitness and Mendelian inheritance. In evolutionary terms, this population genome ensures survival of the species by an optimal balance between fertility and survival, given the characteristics of a specific environment. The stringent selective pressure on our genome (and that of other species) has led the human species to differ from other species with respect to life history traits, such as growth, lifespan and fertility.

The Human Genome Project, which for the first time sequenced the entire genome, has uncovered the considerable genetic variation that exists between human individuals. From the total of 3 billion base pairs that constitute our genome, every one in thousand is polymorphic. A substantial part of these three million polymorphisms explain inter-individual differences in biological processes as they modify the function or quantity of one of the thirty thousand proteins that our genome encodes. From an evolutionary point of view, these inter-individual differences optimizes that ability of the species to survive under present and changing environmental conditions. However, the genetic variation that exists between individuals can also be used as a tool to determine the function of a gene. Each gene is a candidate to influence the length and quality of our lives, but the study of all thirty thousand genes at the same time is expensive and time consuming. Therefore, the identification of specific candidate genes is more efficient.

MODEL ORGANISMS PROVIDE CANDIDATE GENES FOR HUMAN HEALTH AND LONGEVITY

Pathways known to regulate the length of life in model organisms are conserved throughout evolution. It even appeared that point mutations in single genes in model organisms can have substantial effects on survival. For example, it was first discovered that the *C. elegans* daf-2 mutant was long lived (Kenyon et al., 1993). Later it was discovered that the daf-2 gene shows homology to the mammalian genes encoding the Insulin Receptor (IR) and Insulin-like Growth Factor 1 Receptor (IGF-1R) (Kimura et al., 1997), which are conserved throughout evolution. Extended lifespan was then also demonstrated in IR mutants in D. melanogaster (Tatar et al., 2001) and in IR and IGF-1R mutants in mice (Bluher et al., 2003; Holzenberger et al., 2003). Interestingly, these pathways are also conserved in humans.

A widely used approach in model organisms to study the effect of a gene on lifespan is to genetically alter or knock out that gene and observe the effect on lifespan. Similar studies on genetic determinants of lifespan in humans are hampered by technical and ethical obstacles. In contrast to model organisms, humans cannot be genetically altered, and even if this would be possible a lifespan experiment with humans would easily take up to a hundred years.

An alternative approach to identify genetic determinants of healthy longevity in humans is to translate results obtained in model organisms to humans. In the recent past, genomic tools have become available that enable identifying and analyzing the role of conserved pathways and their components in humans. Publicly available databases with polymorphisms in the human genome enable the study of variation of gene function. Furthermore, high throughput genotyping platforms can now generate data at quantities that were as yet unforeseen. These developments have contributed to the increasing use of comparative genomics to disentangle biological pathways and their role in life history traits.

AIM OF THIS THESIS

The present thesis aims to identify candidate genes for healthy longevity in model organisms and to translate results obtained in model organisms to test the role of human genes in determining healthy longevity.

THE LEIDEN 85-PLUS STUDY

The studies in this thesis were carried out using the Leiden 85-Plus Study. The Leiden 85-Plus Study is a population based prospective follow-up study, which consists of two cohorts. In cohort '87 all citizens of Leiden, The Netherlands who were 85 years of age or over on November 1st 1987 were invited to take part. In cohort '97 all citizens of Leiden, The Netherlands were enrolled between September 1997 and September 1999 in the month of their 85th birthday. For the present thesis cohort '97 was used. The proportion of the original birth cohort that has reached the age of 85 is 36% for women and 15% for men. This indicates that the sample under study is selected for survival. It is therefore reasonable to assume that the genotype associated with healthy longevity is enriched in this cohort.

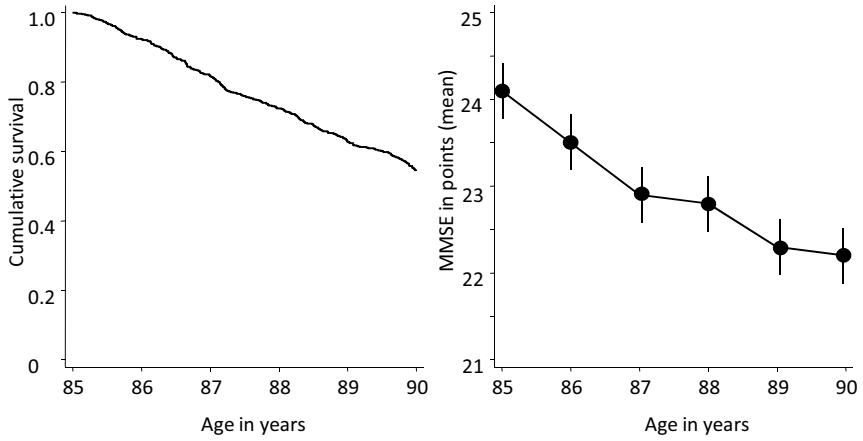


Figure 1.1 *Examples of endpoints in the Leiden 85-plus Study. All participants (n=563) were followed for survival up to the age of ninety (left panel). Various endpoints were measured annually, such as the MMSE score (right panel). Dots represent point estimates of mean MMSE each year, adjusted for gender and level of education. Vertical lines represent 95% confidence intervals.*

Extensive phenotyping was performed at baseline and each year during follow-up until the age of ninety was reached or the participant died. Participants were visited at their place of residence, blood samples were drawn, biometrical measurements were taken, standardized questionnaires were administered regarding health status and functioning. Furthermore, the participants' general practitioner returned questionnaires regarding health status and pharmacy records were retrieved to monitor medication use. Finally, participants were followed for survival, and causes of death were classified into standardized ICD10 codes by specialists in Internal Medicine.

The prospective nature of the Leiden 85-Plus Study, the repeated measurements each year of clinical endpoints (such as cognitive function) together with the high absolute mortality risk in the elderly population makes the study extremely powerful to detect the association of genetic variation with various endpoints (figure 1.1). The response rate (87%) implicates that the findings obtained with this study can be extrapolated to the elderly population at large.

GENES, INTERMEDIATE PHENOTYPES AND ENDPOINTS

In the study of candidate genes for healthy longevity, a recurring approach is used (figure 1.2). First, from studying results obtained in model organisms, or from a genes' function in a biological pathway, a candidate gene is identified. Second, the association between the genotype and the endpoint (or phenotype) of interest is tested. Third, intermediate phenotypes are studied for the association with the genetic variant and the endpoint, in an attempt to explain the biological mechanism that underlies the effect of the genetic variant.

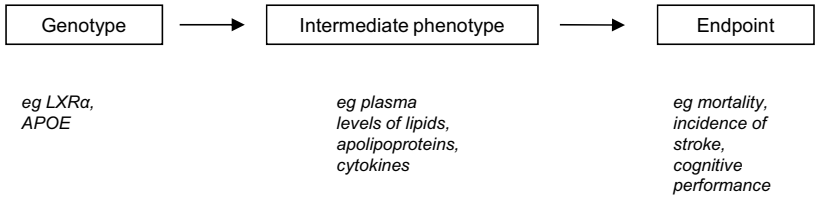


Figure 1.2 *Recurrent model of study outline*

OUTLINE OF THIS THESIS

In **chapter 2** we identify the DAF-12 as an important candidate to modify lifespan in the nematode worm Caenorhabditis Elegans. Human proteins similar to DAF-12 were identified and their biological role was reviewed. The most similar human NHRs are the Vitamin D Receptor and the Liver X Receptor alpha (LXRα). The large number of polymorphisms in the VDR are well described. Therefore, in **chapter 3**, we studied the association of polymorphisms in the VDR with lifespan and disease in old age. No polymorphisms in the LXRα have previously been studied. In **chapter 4** we identified haplotypes of the LXRα and associated them with lifespan and various intermediate phenotypes. An important target gene of LXRα is apolipoprotein E. We thus studied the association of plasma apoE levels with various phenotypes at old age, such as mortality

(**chapter 5**), cognition (**chapter 6**) and stroke (**chapter 7**). In **chapter 8** we studied the association of plasma apoCI (another LXRα target gene) levels with mortality patterns. Finally, in **chapter 9**, we discuss the use of comparative genomics for the identification of evolutionarily conserved mechanisms that preserve human health.

FRAMEWORK

This thesis was carried out within the framework of an “Innovative Oriented Research” (IOP) project entitled “genetic determinants of longevity and disease in old age". The project was a collaboration of biologists, geneticists and physicians and aimed to translate knowledge from model organisms to humans and vice versa. The project set out with the specific aim to generate new insights into control of health and disease in old age. These mechanisms could serve as candidate mechanisms in which interventions would lead to living healthier for longer. Subsidized by the Dutch Ministry of Economic Affairs, there was tight collaboration with industrial partners (Unilever, Numico amongst others) to maximize the opportunity to generate knowledge with the potential to exploit commercially.