A study into genes encoding longevity in humans

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CHAPTER 1

General Introduction

Introduction

The lifespan of an organism is determined by a complex network of environmental-, genetic- and stochastic factors. Each of these components contributes to the wide variability in lifespan between and within species. In recent years, a central question has been to what extent the variability in human life span is related to genetic differences and whether there are common genetic determinants that influence lifespan. To date, we know that 20-30 % of the overall variation in human lifespan is accounted for by genetic factors, which become increasingly important for survival at oldest ages (Herskind et al., 1996; Mitchell et al., 2001; vB et al., 2006). In addition, a number of candidate genes have arisen for the study of longevity in humans, of which the majority has emerged from studies with model organisms.

The most commonly studied model organisms are the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the house mouse *Mus musculus*. These organisms have many advantages for ageing studies, most notably their relatively short life spans, well-characterized biology and completely sequenced genomes, which have allowed rapid progress in the discovery of pathways underlying longevity. The first pathway that was identified was the evolutionarily conserved insulin/IGF1-like signal (IIS) transduction pathway. In *C. elegans*, mutations in the *daf-2* and *age-1* genes, which are related to the mammalian insulin receptor and to the catalytic subunit of phosphatidylinositol-3-kinase (PI-3-kinase), respectively, both lead to increased lifespan (Friedman and Johnson, 1988; Kenyon et al., 1993; Larsen et al., 1995; Morris et al., 1996). In following studies, similar effects were observed for other genes in the IIS pathway and in other pathways involved in metabolic- and physiologic processes, that regulate stress resistance, fertility and genomic maintenance (Christensen et al., 2006; Vijg and Suh, 2005). These findings have provided evidence that individual genes can have major effects on lifespan, but it is largely unknown whether the same genes and processes are also important for the observed variation in human life span. Human genes homologous to the longevity genes identified in model organisms represent relevant candidate genes for the study of longevity in humans.

In addition to the longevity genes identified in model organisms, other candidate genes can de deduced from the biology of human ageing and lifespan. A consistent feature of environmental and genetic factors that influence lifespan is their influence on stress resistance. Most types of stressors are perceived first by the nervous system and the responses of the whole body to such stressors are coordinated by the brain. In humans, the ability to cope with stress and maintain a good mental performance are essential for a long life. Therefore, genes involved in both central nervous system- and peripheral stress responses may play important roles in lifespan determination (Mattson et al., 2002). Yet another set of candidate genes can be derived from human premature ageing syndromes, such as Hutchinson-Gilford syndrome, Werner syndrome, Bloom syndrome, Cockayne's syndrome and Xeroderma pigmentosum. It is unknown whether subtle variations in these genes influence ageing in the population at large. Taken together, different approaches have yielded a number of candidate longevity genes that await testing in humans. Understanding the role of specific genetic factors in the variation of lifespan among humans is central to the understanding of human ageing and lifespan, including exceptional longevity.

The candidate longevity genes

Daf-16 - forkhead transcription factors

The main downstream target of the IIS pathway is the transcription factor dauer formation -16 (*daf-16*), which regulates the expression of numerous downstream genes that mediate stress resistance, innate immunity and metabolic processes (McElwee et al., 2003; Murphy et al., 2003). In *C. elegans*, increased activity of Daf-16 has been associated with increased lifespan (Kenyon et al., 1993; Kimura et al., 1997). In contrast, mutations in the *daf-16* gene have been shown to suppress the life-extending effects of decreased insulin signaling (Lin et al., 1997). These data indicate that *daf-16* is negatively regulated by insulin signaling and is the major downstream effector of the IIS pathway. In mammals, the *daf-16* gene homologues are forkhead/winged-helix transcription factors (FOXOs) of which to date, four different family members have been identified: *FOXO1a*, *FOXO3a*, *FOXO4* and *FOXO6* (Anderson et al., 1998; Biggs et al., 2001; Jacobs et al., 2003). From these, cellular functions have been described best for FOXO1a and FOXO3a. Both of these genes are involved in a variety of cellular processes, including metabolism, cell differentiation, cell cycle arrest and DNA repair. In addition, FOXO1a has been specifically implicated in mediating the effects of insulin on hepatic glucose production (Altomonte et al., 2003; Barthel et al., 2005; Nakae et al., 2002) while FOXO3a has been specifically implicated in female fertility through suppression of follicular activation (Castrillon et al., 2003).

Daf-12 - liver X receptors and vitamin D receptor

Dauer formation-12 (*Daf-12*) gene belongs to the nuclear hormone receptor (NHR) superfamily, a large and diverse family of transcription factors (Laudet, 1997), and it has been placed at the convergence of several signal transduction pathways, including the IIS pathway. Similar to other Daf proteins in *C. elegans*, Daf-12 regulates dauer diapause, developmental timing and metabolism in response to environmental signals (Rottiers and Antebi, 2006). Mutations in the daf-12 gene can result in dauer defective or dauer constitutive worms, which are short- and long-lived, respectively. In addition, it has been shown that the long-lived phenotype of germline-ablated worms depends, besides on daf-16, also on daf-12 (Hsin and Kenyon, 1999). In humans, the closest *daf-12* homologues are the liver X receptors (*LXRA* and *LXRB*) and the vitamin D receptor (*VDR*) (Mooijaart et al., 2005). These genes belong to NHR super-family, but have distinct functions. The LXRs are mainly involved in lipid metabolism and -transport (Peet et al., 1998; Tontonoz and Mangelsdorf, 2003), whereas the VDR is involved in diverse functions that include bone metabolism, cellular proliferation and differentiation, immunomodulation and neuroprotection (Lin and White, 2004).

Sir2 - sirtuins

The Sirtuins represent an evolutionarily conserved family of Silent Information Regulator 2 (Sir2) NAD-dependent protein deacetylases that interact with and influence the activity of vari-

ous transcription factors and co-regulators (Bordone and Guarente, 2005). Increased expression of the *Sir2* gene, either due to an extra copy of the gene or to caloric restriction, has been shown to prolong lifespan in various model organisms (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001; Wood et al., 2004). In mammals, there are seven *Sir2* homologues (*SIRT1-7*), of which *SIRT1* is the most closely related to *Sir2* (Frye, 2000). Studies with mouse models have shown that in response to environmental signals, SIRT1 regulates glucose and fat metabolism, stress resistance and cell survival (Bordone and Guarente, 2005; Cohen et al., 2004; Picard et al., 2004; Yang et al., 2006). Some of the target genes through which SIRT1 exerts these effects include the *FOXOs*, *p53* and *PPAR-gamma* (Leibiger and Berggren, 2006).

Mineralocorticoid- and glucocorticoid receptor

The mineralocorticoid receptor (*MR*) and glucocorticoid receptor (*GR*) genes, which belong to the NHR family, and are activated by cortisol to regulate metabolism, inflammation, and immunity (Meijer et al., 2006). Cortisol is the primary active stress hormone that mediates counter-responses to stress, aimed to re-establish homeostasis and coordinate behavioral adaptations (de Kloet et al., 2005). By targeting many genes through the MR and GR, cortisol functions in a binary fashion, and serves as a master switch in the control of neuronal and network responses that underlie behavioral adaptation. Various studies have shown that stress-responsiveness is highly variable among human subjects, and an inadequate stress-response increases vulnerability for disease. Changes in the stress hormone system have been shown to play a role in cognitive impairment (Lupien et al., 2005) and in the development of depression (Belanoff et al., 2001; Holsboer, 2000). The ability to cope with stress and maintain a good mental performance are essential for a long life, which place the MR and GR genes on the list of candidate genes important for human longevity.

WRN

The *WRN* gene encodes a nuclear protein with both helicase and exonuclease activities (Liu et al., 1999; Morozov et al., 1997; Mushegian et al., 1997). The WRN protein is capable of a multitude of functions and is involved in DNA replication, repair, recombination, transcription and/or a combination of these events. Loss-of function mutations in the *WRN* gene lead to Werner syndrome (WS), which is a segmental progeroid disorder with an autosomal recessive pattern of inheritance. Patients with WS exhibit a number of symptoms that resemble premature ageing. Characteristic clinical features of the syndrome include diabetes, osteoporosis, vascular diseases and a high incidence of malignant neoplasms (Martin, 1978; Salk, 1982). Since mutations in the *WRN* gene lead to accelerated ageing, it has been reasoned that common polymorphism in the *WRN* gene could contribute to the differences in the prevalence of disease and lifespan in the general population.

Candidate-gene-based association studies

Candidate-gene-based association studies assess correlations between genetic variants in the candidate gene and differences in the trait of interest on a population scale. In analyzing the role of a candidate gene in humans, the association between DNA variants in, around and nearby the candidate gene and the trait of interest are analyzed. The DNA variants investigated most often are single nucleotide polymorphisms (SNPs). Until recently, there were relatively few SNPs available for study, but advances in the past two decades have identified millions of such polymorphisms. The availability of a large number of SNPs in public databases has facilitates the selection of genetic variants for association studies. There are two approaches that can be used for the identification of DNA variants related to the phenotype of interest: the direct- and the indirect association approach (Figure 1) (Carlson et al., 2004; Cordell and Clayton, 2005).

Direct association studies make use of polymorphisms which are themselves putative causal variants (Carlson et al., 2004; Cordell and Clayton, 2005). This type of study is the easiest to analyze and the most powerful, but its difficulty is the identification of candidate polymorphisms. A mutation in a codon, which leads to an aminoacid change, is a candidate causal variant. However, it is likely that many causal variants responsible for heritability of common complex disorders will be non-coding. For example, such variants may cause variation in gene regulation and expression, or differential splicing. The exponential increase in annotation of common variants has generated a catalog of variants of which we know nothing about the function for the vast majority. Thus, for the direct association approach prior knowledge of functionality of the SNPs has to be first gained through functional studies.

The second, indirect approach, does not rely on the functionality of the polymorphisms but on linkage disequilibrium (LD) between a disease susceptibility allele and either a single marker allele or a multilocus haplotype (Carlson et al., 2004; Cordell and Clayton, 2005; Newton-Cheh and Hirschhorn, 2005). Several polymorphisms are commonly selected from a candidate gene and the polymorphisms under study serve as surrogates for the causal locus. Much recent meth-

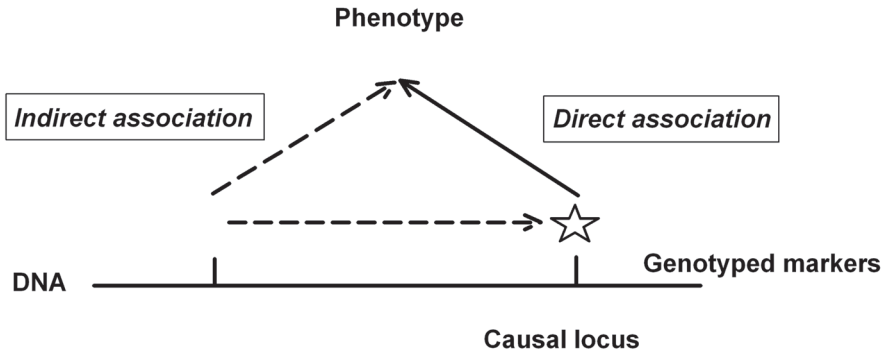

Figure 1. The direct- and indirect association approach

odological work has been conducted to optimize this indirect approach, including the investigation of haplotype-block structure and techniques for selecting haplotype-tagging SNPs. The systematization of the indirect approach is the aim of the HapMap Project (The International HapMap Consortium, 2005). In this thesis, both the direct- and indirect association strategies were used.

The Leiden 85-plus Study

All studies presented in this thesis were performed within the Leiden 85-plus Study. The Leiden 85-plus Study is a prospective population-based study, in which all 85 year old or older inhabitants of the city Leiden, The Netherlands, were invited to take part. The study population consists of two cohorts, cohort '87 and '97. The Medical Ethical Committee of the Leiden University Medical Centre approved the study.

Cohort '87

On December 1, 1986, the community of Leiden in the Netherlands had 105 000 inhabitants, of whom 1258 (1.2 %) were 85 years and older. Among these oldest old, a population-based prospective follow-up study was initiated to assess the association of HLA antigens with human lifespan (Izaks et al., 1997; Lagaay et al., 1992). During an assessment, which lasted from December 1986 to March 1989, 221 participants died before they could be visited. From the remaining 1037 people, 977 (94 %) agreed to participate in study (Weverling-Rijnsburger et al., 1997). All participants were interviewed at their place of residence by an internist experienced in geriatric medicine. After oral informed consent was obtained, a Mini-Mental State Examination (MMSE) and General Health Questionnaire (GHQ-12) were administered to detect cognitive impairment. The Dutch version of the Geriatric Mental State Schedule (GMS) was used to diagnose psychiatric disorders according to Diagnostic and Statistical Manual of Mental Disorders III (DSM-III) criteria. A complete medical history was taken with special emphasis on cardiovascular disease, diabetes mellitus, and other chronic disorders together with information about the living situation and demography. When it was not possible to get reliable information from the participant, a family member or a caretaker was asked to provide the information. In addition, diastolic- and systolic blood pressure, and glucose levels were measured. Blood samples were taken at their homes, according to predefined protocols under non-fasting conditions. After isolation of the leucocytes for HLA typing, which was the primary goal of the study, the remaining serum was available for other laboratory measurements. For DNA extraction, sufficient cell material was available for 682 participants.

Cohort '97

Between September 1997 and September 1999, 705 inhabitants of the city Leiden, in The Netherlands, reached the age of 85 years, and in the month after their 85th birthday, they were asked to participate in the Leiden 85-plus Study. Of the 705 eligible subjects 14 died before they

could be enrolled and 92 refused to participate, resulting in a cohort of 599 subjects (85 %) who were enrolled (der Wiel et al., 2002). All the study participants were visited at their place of residence, where face-to-face interviews were conducted, cognitive testing was performed, and a venous blood sample was drawn. Of the 599 participants, a venous blood sample was available for 563 participants. During the main interview with participants, global cognitive function was assessed with the Mini-Mental State Examination (MMSE), attention with the Stroop Test (Klein et al., 1997), processing speed with the Letter Digit Coding Test (LDT) (Houx et al., 2002), and memory with the 12-Word Learning Test, which assesses immediate recall (WLTI) and delayed recall (WLTD)(Brand and Jolles, 1985). The prevalence of depressive symptoms was assessed with the 15-item Geriatric Depression Scale (GDS-15) (De Craen et al., 2003). All participants were visited annually for re-measurement of cognitive functioning and depressive symptoms during a mean follow-up period of 4.2 years. For all subjects socio-demographic characteristics such as gender, marital state, and type of housing were available from the municipal registry. Informed consent was obtained from all participants, or in case of severe cognitive impairment, from their guardian.

Follow-up of mortality

All participants of the cohort '87 and '97 were followed for mortality until August 1 2005. Primary causes of death were obtained from the Dutch Central Bureau of Statistics and categorized according to the 10th International Classification of Diseases (ICD-10). At the date of censoring, August 1 2005, 681 (99 %) participants of the cohort '87 and 320 (57 %) participants of the cohort '97 had died. The most frequent primary cause of death in the participants of the Leiden 85-plus Study was death due to cardiovascular diseases (Table 1), as is the case in the general population.

Table 1. The causes of death in the Leiden 85-plus Study

	The Leiden 85-plus Study		
	Cohort '87 (n=682)	Cohort '97 (n=563)	Combined (n=1245)
All-cause mortality*	681 (99 %)	320 (57 %)	1001 (80 %)
CVD	277 (40 %)	129 (40 %)	406 (41 %)
Cancer	101 (15 %)	61 (19 %)	162 (16 %)
Infectious disease	65 (10 %)	20 (6 %)	85 (8 %)
Other	237 (35 %)	109(34 %)	346 (35 %)

*A cause of death could not be obtained for one participant from both cohort '87 and cohort '97; CVD – cardiovascular disease

Outline of the thesis

The general objective of the thesis was to test the impact of the most prominent longevity candidate genes on the prevalence of age-related diseases and lifespan in a population-based prospective study of the oldest old. The thesis consists of ten chapters, of which in **chapter 1** a general introduction to the thesis is given. In the following chapters, the effect of genetic variance in the evolutionarily conserved genes *FOXO1* and *FOXO3a* (**chapter 2**), *LXR* (**chapter 3**), *VDR* (**chapter 4**) and *SIRT1* (**chapter 5**) on the prevalence of age-related diseases and lifespan was examined. In **chapter 6**, the influence of cortisol levels and of polymorphisms in the mineralocorticoid receptor (*MR*) and glucocorticoid receptor (*GR*) genes on mental performance and on the prevalence of depressive symptoms in old age was assessed. The influence of polymorphisms in the *GR* gene and in the *WRN* gene on the prevalence of age-related diseases and lifespan were studied in **chapter 7** and in **chapter 8**, respectively. In **chapter 9**, the results are summarized and discussed, and the main conclusions are drawn. The last chapter of the thesis, **chapter 10**, contains a summary in Dutch of the thesis.

The research presented in 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", subsidized by the Dutch Ministry of Economic Affairs (grant number IGE010114). This project brought together medical doctors, evolutionary biologist, geneticists and bioinformaticians with the aim to identify mechanisms that determine longevity and disease in old age. In addition there was tight collaboration with industrial partners to maximize the opportunity to generate knowledge with the potential to exploite commercially.

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