SIRT1 Gene, Age-Related Diseases, and Mortality: The Leiden 85-Plus Study

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The (Silent Information Regulator 2) Sir2 gene has been shown to regulate the life span of several model organisms. In mammals, the evolutionarily conserved homologue (Sirtuin 1) SIRT1 regulates neuroprotection, metabolism, and cell survival in response to stress. Based on these data, we hypothesized that SIRT1 might influence the prevalence of age-related diseases and modify the life span of humans. In order to test this, we genotyped five single nucleotide polymorphisms (SNPs) in 1245 participants of the population-based Leiden 85-plus Study. SIRT1 haplotypes were assessed and tested for association with the risks of mortality, metabolic profile, age-related diseases, and cognitive functioning. These analyses revealed a trend for lower cardiovascular mortality for haplotype 2 and rs3758391 SNP carriers. In further analyses, this trend was not supported by intermediate phenotypes, albeit the rs3758391 T-allele carriers had better cognitive functioning. In conclusion, our results indicate a role for SIRT1 in cognitive functioning, but the role in life span remains to be elucidated.

Increased expression of Sir2 (Silent Information Regulator 2) either due to an extra copy of the gene or to caloric restriction, prolongs life span in various model organisms (1–3). In mammals, there are seven Sir2 homologues, of which SIRT1 (Sirtuin 1) is the most similar to Sir2 (4,5). In response to environmental signals, SIRT1 regulates metabolism and cell survival in various types of mammalian cells (6–8). To date, it is unknown whether SIRT1 influences the prevalence of age-related diseases and modifies the life span of humans.

SIRT1 is an NAD+ -dependent (nicotinamide adenine dinucleotide) protein deacetylase (9), and it regulates metabolism and cell survival through influencing gene silencing and the activity of various transcription factors and coregulators (6,10). It has been shown that activation of SIRT1 increases glucose tolerance and enhances insulin response to glucose in pancreatic β cells (11–13). Increased SIRT1 activity also promotes hepatic gluconeogenesis and inhibits glycolysis via peroxisome proliferator-activated receptor γ coactivator 1-α (PGC1-α) during fasting (14). Furthermore, SIRT1 has an effect on fat metabolism, via inhibition of peroxisome proliferator-activated receptor γ (PPARγ) (15). These findings suggest that increased SIRT1 activity results in a favorable metabolic profile, with decreased prevalence of diabetes and cardiovascular diseases. In addition, the role of SIRT1 in providing resistance to damage- or stress-induced apoptosis may help to preserve organ function over time, although by doing so it may promote cancer (16,17). Recent evidence also suggests a role for SIRT1 in neuroprotection and neurodegenerative disorders (18–20). Altogether, SIRT1 could influence life span in several ways. The involvement of SIRT1 in human life span has been previously studied in a case–control study of elderly and young persons (21). No differences in SIRT1 allele and haplotype frequencies were observed between these groups. This finding, however, does not exclude the possibility that SIRT1 gene has an influence on human physiology and life span.

The aim of this study was to analyze the association between genetic variation in the SIRT1 gene and all-cause and cause-specific mortalities. In addition, metabolic profile, prevalence of age-related diseases, and cognitive functioning were tested in the participants of the prospective population-based Leiden 85-plus Study.

Participants and Methods

Study Population

The Leiden 85-plus Study is a prospective, population-based study, in which all 85-year-old or older inhabitants of the city of Leiden, The Netherlands, were invited to take part. The study design and data collection have been described elsewhere (22,23). The study population consists of two cohorts, cohort ’87 and ’97, and all the study participants are of Caucasian origin. Cohort ’87 includes 977 participants 85 years old or older, enrolled between 1987 and 1989 (22). Cohort ’97 consists of 599 participants, all members of the 1912–1914 birth cohorts, who were enrolled in the month of their 85th birthday between 1997 and 1999 (23). DNA was available for 682 participants from cohort ’87 and for 563 participants from cohort ’97. All
Participants were followed for mortality until August 1, 2005, with a mean follow-up period of 4.4 years. 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). The Medical Ethical Committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all participants.

**Metabolic Profile in Cohort '97**

Hemoglobin A1c (HbA1c), triglycerides, C-reactive protein (CRP), and high-density lipoprotein (HDL) cholesterol concentrations were determined in serum using fully automated analyzers (Hitachi 747 and 911; Hitachi Ltd., Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol was estimated with the Friedewald equation (24).

**Cardiovascular Pathologies and Diabetes in Cohort '97**

The prevalence and number of cardiovascular pathologies and diabetes were obtained from the participants' general practitioner or nursing home physician. For cardiovascular pathologies, electrocardiograms also were recorded (25). Cardiovascular pathologies were classified as myocardial infarction, myocardial ischemia, stroke, arterial surgery, or intermittent claudication (26). Participants were classified as having diabetes when they met at least one of the following criteria: (i) history of diabetes obtained from the general practitioner or the participant's treating physician; (ii) use of sulfonylurea, biguanide, or insulin, based on information from the practitioner's pharmacist; or (iii) nonfasting glucose of ≥11.1 mmol/L.

**Cognitive Function and Depressive Symptoms in Cohort '97**

Overall cognitive functioning was measured with the Mini-Mental State Examination (MMSE) (27). From specific domains of cognitive functioning, attention was assessed with the Stroop Test (28), processing speed with the Letter Digit Coding Test (LDT) (29), and memory with the 12-Word Learning Test, which assesses immediate recall (WLTI) and delayed recall (WLTD) (30). The prevalence of depressive symptoms was assessed with the 15-item Geriatric Depression Scale (GDS-15) (31). The tests assessing specific domains of cognitive functioning could not be administered to 92 participants because of severe cognitive impairment (MMSE score ≤ 18 points). All participants were visited annually for remeasurement of cognitive functioning and depressive symptoms during a mean follow-up period of 4.2 years. In addition to the specific tests being readministered, a composite cognitive score was calculated by converting the scores of the individual tests (Stroop Test, LDT, WLTI, and WLTD) into a z score [(individual level – mean level)/SD], and computing the average.

**SNP Selection and Genotyping**

The single nucleotide polymorphisms (SNPs) were selected from the public database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) to cover the SIRT1 gene region (GeneID: 23411) in equally spaced intervals. The minor allele frequencies (MAF) of the polymorphisms had to be > 5%. The selected SNPs were rs3758391 (promoter), rs3740051 (promoter), rs2236319 (intron), rs2273773 (exon), and rs3818291 (intron). All these SNPs were genotyped using the MassArray platform, according to the protocols of the manufacturer (Sequenom Inc., San Diego, CA).

**Statistical Analysis**

The program Haploview (32) was used to estimate the SNPs’ allele frequencies, test the genotypes for Hardy–Weinberg equilibrium, and estimate pairwise linkage disequilibrium (LD) between the polymorphisms. Haplotypes and haplotype frequencies were calculated using SNPHAP software (http://www-gene.cimr.cam.ac.uk/clayton/software). The posterior probabilities of pairs of haplotypes per participant, as estimated by the SNPHAP, were used as weights in all analyses. The haplotype analyses approach used in this study assumes an additive effect of the haplotypes, and details of this approach have been described elsewhere (33). CRP, triglyceride, and HbA1c levels were not normally distributed and were ln-transformed. All-cause and cause-specific mortality risks with 95% confidence intervals (CI) were calculated with the Cox proportional hazard model, using left censoring to correct for the delayed entry into the risk set according to age. Associations between haplotypes and metabolic profile were analyzed using the general linear model. Differences in the prevalence of cardiovascular pathologies and diabetes between the haplotypes were tested using binary logistic regression. The associations between cognitive functioning, depressive symptoms, and haplotypes were tested with the linear mixed model. All analyses were sex adjusted, except the analyses of cognitive functioning and depressive symptom, which were additionally adjusted for education. The analyses were performed with STATA (version 9.0; StataCorp LP, College Station, TX) and SAS (version 8.2; SAS Institute Inc., Cary, NC) statistical software.

**RESULTS**

All 1245 participants of the Leiden 85-plus Study were genotyped for the five SIRT1 polymorphisms (Table 1). The

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**Table 1. Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cohort ’87</th>
<th>Cohort ’97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>682</td>
<td>563</td>
</tr>
<tr>
<td>Age (median, IQR)</td>
<td>89 (88–92)</td>
<td>85 (–)</td>
</tr>
<tr>
<td>Female (n, %)</td>
<td>491 (72%)</td>
<td>375 (67%)</td>
</tr>
<tr>
<td>Polymorphisms*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3758391 (C/T)</td>
<td>0.33</td>
<td>0.36</td>
</tr>
<tr>
<td>rs3740051 (A/G)</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>rs2236319 (A/G)</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>rs2273773 (T/C)</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>rs3818291 (G/A)</td>
<td>0.13</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Notes: *Minor allele frequency.
IQR = interquartile range.
genotype frequencies of the SNPs were in Hardy–Weinberg equilibrium, and the allele frequencies were similar between the two elderly cohorts (Table 1). The polymorphisms were in strong LD ($D^2$ 0.97–1.00), and gave rise to five different haplotypes of which four were common (frequencies $>5\%$) and cumulatively accounted for 99.9\% of the haplotypes (Figure 1).

All-cause and cause-specific mortality risks were assessed after a mean follow-up period of 4.4 years. During that time, 1001 (80\%) of the 1245 participants had died. Of these, 406 (41\%) had died due to cardiovascular disease, 162 (16\%) due to cancer, and 431 (43\%) due to other causes. The cause of death was unknown for two participants. The mortality risk analyses revealed a trend for lower cardiovascular mortality (hazard ratio [HR] 0.82, 95% CI, 0.66–1.01; $p = 0.062$) for haplotype 2 carriers, compared to the reference haplotype (Figure 2). A similar trend was observed in the two Leiden 85-plus Study cohorts separately (data not shown). For the other haplotypes, no differences in all-cause or cause-specific mortality risks were observed (Figure 2).

In order to study further the role of SIRT1, we analyzed the associations between SIRT1 haplotypes and metabolic profile, prevalence of cardiovascular diseases, and diabetes. The data on these endpoints were available for 563 participants of the cohort ’97. At baseline, no associations between the SIRT1 haplotypes and various metabolic profile parameters were observed, except for LDL cholesterol and haplotype 3. These haplotype carriers had 0.18 mmol/L lower (95\% CI, −0.34 to −0.02; $p = 0.030$) LDL levels compared to the reference haplotype. In contrast, none of the SIRT1 haplotypes were associated with the prevalence of cardiovascular pathologies or diabetes. For haplotype 2 carriers, nonsignificant trends for lower prevalence of arterial surgery (odds ratio [OR] 0.82, 95\% CI, 0.41–1.65; $p = 0.574$) and intermittent claudication (OR 0.76, 95\% CI, 0.36–1.60; $p = 0.471$) were observed (Table 2).

In the cohort ’97, cognitive functioning and the prevalence of depressive symptoms were assessed at baseline at age 85 years, and were reexamined annually during a mean follow-up period of 4.2 years. Compared to the reference haplotype, there were no differences in cognitive functioning or in prevalence of depressive symptoms between the SIRT1 haplotypes (data not shown).

In addition to haplotype analyses, univariate analyses with the individual polymorphisms were performed. From the five polymorphisms, associations with only one (rs3758391), which also resides in the haplotype 2, were observed. Heterozygous (HR 0.77, 95\% CI, 0.62–1.00; $p = 0.018$) but not homozygous (HR 1.01, 95\% CI, 0.73–1.39; $p = 0.965$) carriers of rs3758391 T allele had lower cardiovascular mortality risks. These differences were not attributable to changes in metabolic profile or in prevalence of age-related diseases (data not shown). In contrast, homozygous but not heterozygous carriers of the rs3758391 T allele performed better on all tests measuring cognitive functioning (Table 3). These differences were most pronounced for immediate memory (2.26 points, 95\% CI, 0.62–3.89; $p = 0.007$) and for delayed memory (1.06 points, 95\% CI, 0.29–1.84; $p = 0.007$) (Table 3).

**Discussion**

In this study, we tested the role of SIRT1 in age-related diseases, cognitive functioning, and mortality in humans. The analyses of SIRT1 haplotypes revealed a trend for decreased cardiovascular mortality for haplotype 2 carriers, and for the
rs3758391 (which resides in the haplotype 2) SNP carriers. None of these, however, were associated with metabolic profile or cardiovascular pathologies. In contrast, carriers of the rs3758391 polymorphism performed better than non-carriers did on tests measuring cognitive functioning.

A specific role of SIRT1 in cell survival and in the development of cancer has been proposed (34–36). In this study, we found no associations between SIRT1 haplotypes and cancer mortality, but we observed a trend for lower cardiovascular mortality for haplotype 2 carriers. This trend was observed in the combined, but also in the separate cohorts. Altogether, these observations are in accordance with the results from cell culture studies, in which a protective effect of SIRT1 on cardiac myocytes has been demonstrated (37,38). In addition, SIRT1 appears to be important for the development of heart, as Sirt1−/− knockout mice presented cardiac abnormalities (39,40). Based on these data, the lower cardiovascular mortality in the haplotype 2 carriers in our study population is in line with the expected functions of SIRT1. The lower risk for cardiovascular mortality and the SIRT1 haplotype 2 could have arisen either due to other mechanisms or due to chance. It might also be that the beneficial effects of SIRT1 only appear in acute disease states, thereby decreasing the severity of outcomes from crisis events. This mode of action is consistent with the effects of SIRT1 on apoptosis.

Besides mortality and various intermediate phenotypes, we tested the role of SIRT1 in cognitive functioning and in prevalence of depressive symptoms. The involvement of SIRT1 in neurophysiological functioning has been discovered recently. Several studies have linked the SIRT1 protein and its biological activator, resveratrol, to axonal protection and survival of neurons (18–20). Axonal degeneration often precedes the death of neuronal cell bodies in neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease (41). As a result, impairments in cognitive functioning occur. In this study, we found no associations between SIRT1 haplotypes and cognitive functioning and depressive symptoms. In contrast, a promoter polymorphism (rs3758391), which is the only variant allele in the haplotype 2, was associated with better cognitive functioning. From the specific domains of cognitive functioning, memory was the best preserved. These data, together with the evidence from recent literature, support a possible role of SIRT1 in the brain.

Our results are partly in accordance with a recent case-control study, in which no associations between SIRT1 polymorphisms/haplotypes and life span were found (21). In both studies, five polymorphisms from the SIRT1 gene were analyzed, although only two were the same between the

Table 2. Sirtuin 1 (SIRT1) Haplotypes, Prevalence of Cardiovascular Diseases (CVD), and Diabetes at Baseline in the Leiden 85-Plus Study Cohort ‘97 (n = 563)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Haplotype 1 OR (95% CI)</th>
<th>Haplotype 2 OR (95% CI)</th>
<th>Haplotype 3 OR (95% CI)</th>
<th>Haplotype 4 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD total (n = 365)*</td>
<td>1 (Reference)</td>
<td>1.17 (0.83–1.64)</td>
<td>1.11 (0.77–1.60)</td>
<td>0.84 (0.53–1.33)</td>
</tr>
<tr>
<td>Myocardial infarction (n = 137)</td>
<td>1 (Reference)</td>
<td>0.99 (0.69–1.44)</td>
<td>0.90 (0.60–1.36)</td>
<td>0.98 (0.58–1.67)</td>
</tr>
<tr>
<td>Myocardial ischemia (n = 286)</td>
<td>1 (Reference)</td>
<td>1.02 (0.74–1.40)</td>
<td>1.08 (0.77–1.51)</td>
<td>0.91 (0.58–1.44)</td>
</tr>
<tr>
<td>Stroke (n = 57)</td>
<td>1 (Reference)</td>
<td>1.07 (0.61–1.88)</td>
<td>1.14 (0.67–1.95)</td>
<td>0.90 (0.37–2.23)</td>
</tr>
<tr>
<td>Arterial surgery (n = 37)</td>
<td>1 (Reference)</td>
<td>0.82 (0.41–1.65)</td>
<td>0.98 (0.48–1.97)</td>
<td>0.75 (0.27–2.08)</td>
</tr>
<tr>
<td>Intermittent claudication (n = 36)</td>
<td>1 (Reference)</td>
<td>0.76 (0.36–1.60)</td>
<td>0.92 (0.43–2.00)</td>
<td>0.78 (0.29–2.09)</td>
</tr>
<tr>
<td>Diabetes (n = 92)</td>
<td>1 (Reference)</td>
<td>1.37 (0.89–2.11)</td>
<td>1.46 (0.93–2.28)</td>
<td>0.79 (0.40–1.55)</td>
</tr>
</tbody>
</table>

Notes: *Participants with one or more cardiovascular pathologies. OR = odds ratio; CI = confidence interval.

rs3758391 (which resides in the haplotype 2) SNP carriers. None of these, however, were associated with metabolic profile or cardiovascular pathologies. In contrast, carriers of the rs3758391 polymorphism performed better than non-carriers did on tests measuring cognitive functioning.

A specific role of SIRT1 in cell survival and in the development of cancer has been proposed (34–36). In this study, we found no associations between SIRT1 haplotypes and cancer mortality, but we observed a trend for lower cardiovascular mortality for haplotype 2 carriers. This trend was observed in the combined, but also in the separate cohorts. Altogether, these observations are in accordance with the results from cell culture studies, in which a protective effect of SIRT1 on cardiac myocytes has been demonstrated (37,38). In addition, SIRT1 appears to be important for the development of heart, as Sirt1−/− knockout mice presented cardiac abnormalities (39,40). Based on these data, the lower cardiovascular mortality in the haplotype 2 carriers in our study population is in line with the expected functions of SIRT1. The lower risk for cardiovascular mortality implies that these SIRT1 haplotype carriers might suffer less from cardiovascular diseases than non-carriers. In order to test that implication, we analyzed the prevalence of various cardiovascular pathologies dependent on SIRT1 haplotypes. However, no associations were found, and also the parameters of metabolic profile, which underlie atherosclerosis, did not differ. For the latter, a beneficial profile was expected for the SIRT1 haplotype 2 carriers. The lack of a consistent risk profile suggests that the association between the lower cardiovascular mortality and the SIRT1

Table 3. Sirtuin 1 (SIRT1) rs3758391 Single Nucleotide Polymorphism (SNP), Cognitive Functioning, and Depressive Symptoms During Mean Follow-Up Period of 4.2 Years in the Leiden 85-Plus Study Cohort ‘97 (n = 563)

<table>
<thead>
<tr>
<th>Mental Performance</th>
<th>rs3758391</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC Mean (SE)</td>
<td>CT Difference (SE)</td>
<td>TT Difference (SE)</td>
<td>p Trend</td>
</tr>
<tr>
<td>Composite cognitive score</td>
<td></td>
<td>–0.23 (0.06)</td>
<td>0.02 (0.08)</td>
<td>0.28 (0.11)*</td>
<td>.031*</td>
</tr>
<tr>
<td>Global cognitive function</td>
<td></td>
<td>22.7 (0.43)</td>
<td>0.24 (0.59)</td>
<td>0.63 (0.83)</td>
<td>.443</td>
</tr>
<tr>
<td>(points)</td>
<td></td>
<td>87.0 (2.12)</td>
<td>1.67 (2.90)</td>
<td>–2.28 (4.10)</td>
<td>.823</td>
</tr>
<tr>
<td>Attention (seconds)</td>
<td></td>
<td>15.7 (0.46)</td>
<td>–0.11 (0.63)</td>
<td>0.97 (0.88)</td>
<td>.414</td>
</tr>
<tr>
<td>Processing speed (digits)</td>
<td></td>
<td>20.1 (0.43)</td>
<td>–0.10 (0.59)</td>
<td>2.26 (0.83)*</td>
<td>.035*</td>
</tr>
<tr>
<td>Immediate memory (pictures)</td>
<td></td>
<td>6.86 (0.20)</td>
<td>0.02 (0.28)</td>
<td>1.06 (0.39)*</td>
<td>.029*</td>
</tr>
<tr>
<td>Delayed memory (pictures)</td>
<td></td>
<td>2.97 (0.18)</td>
<td>0.18 (0.25)</td>
<td>–0.17 (0.36)</td>
<td>.912</td>
</tr>
<tr>
<td>Depressive symptoms (points)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

Notes: *p < .05. SE = standard error.
studies. However, besides analyzing individual polymorphisms, we calculated haplotypes and tested their association with various phenotypes. The SIRT1 gene is embedded in a region of strong LD (Supplementary Figure 1), and a haplotype-based approach enables us to capture the majority of the genetic variation in the SIRT1 gene. As no associations with intermediate phenotypes were observed, we reason that the association between the rs3758391 SNP and cognitive functioning has arisen due to polymorphisms in LD with those analyzed in this study. These SNPs could reside in neighboring genes (DNAJC12 in 5' and HERC4 in 3') or in the regulatory regions of the SIRT1 gene. We speculate that functional variability in the SIRT1 gene itself is constrained because it plays diverse but essential roles in human physiology.

In mammals, the Sir2 gene has several homologues (42), and perhaps one or more of the other SIRT family members (SIRT1–7) have bigger influences on life span. From this point of view, the SIRT3 gene (which encodes a mitochondrial protein) has been implicated to play a role in human longevity (43,44). In addition, recent evidence suggests a similar role for SIRT6, as Sirt6-deficient mice displayed genomic instability and a premature aging-like phenotype (45). Therefore, the analyses of other SIRT family members might shed more light on the regulation of human life span.

The strengths of the study include the possibility of estimating several phenotypes in one cohort, and the prospective analyses with a high number of deaths during follow-up. A limitation of the study is the lack of data on the activity or levels of SIRT1, which would reflect the functionality of the polymorphisms analyzed. In addition, considering the number of tests performed, adjustment for multiple testing would eliminate all the statistically significant associations observed. Consequently, the results of this study are not exhaustive.

**Conclusion**

The results of this study provide evidence for a role of SIRT1 in cognitive functioning, but due to the lack of associations with intermediate phenotypes, the influence of genetic variation in the SIRT1 gene on life span remains to be elucidated.
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