Family matters: a genealogical inquiry into the familial component of longevity

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CHAPTER 5

LONGEVITY AROUND THE TURN OF THE 20TH CENTURY:

LIFE-LONG SUSTAINED SURVIVAL ADVANTAGE FOR PARENTS OF TODAY'S NONAGENARIANS

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Abstract

Members of longevous families live longer than individuals from similar birth cohorts and delay/escape age related diseases. Insight into this familial component of longevity can provide important knowledge about mechanisms protecting against age-related diseases. This familial component of longevity was studied in the Leiden Longevity Study which consists of 944 longevous siblings (participants), their parents (N=842), siblings (N=2302), and spouses (N=809). Family longevity scores were estimated to explore whether human longevity is transmitted preferentially through the maternal or paternal line. Standardized mortality ratio's (SMRs) were estimated to investigate whether longevous siblings have a survival advantage compared to longevous singletons and we investigated if parents of longevous siblings harbor a life-long sustained survival advantage compared to the general Dutch population by estimating lifetime SMRs (L-SMRs). We found that sibships with long-lived mothers and non-long-lived fathers had 0.41 (P=0.024) less observed deaths than sibships with long-lived fathers and non-long-lived mothers and 0.48 (P=0.008) less observed deaths than sibships with both parents non-long lived. Participants had 18.6% less deaths compared to matched singletons and parents had a life-long sustained survival advantage (L-SMR=0.510 and 0.688). In conclusion, genetic longevity studies may incorporate the maternal transmission pattern and genes influencing the entire life-course of individuals.

Introduction

The average human life expectancy steadily increased over the last 200 years in industrialized countries, with record life expectancy increasing from 43/45 years in 1840 to 79/85 years in 2015 for males and females respectively[1]. Until 1950 the average increase in life expectancy could mainly be attributed to improved living conditions and better healthcare, causing a decrease in childhood and early life mortality[2]. After 1950 the average life expectancy increased due to a delay of mid and late-life mortality[3–6]. Despite the average increase in life expectancy in the industrialized countries, significant individual differences in lifespan, defined as age at death, exist[7,8]. In fact, a small group of individuals is able to survive into exceptionally old ages. This longevity capacity clusters within families[9–11] and on top of that, members of such long-lived families seem to delay or even escape age-related disease[12–15]. Hence, research into long-lived families plays a key role in gaining knowledge about how to prevent age related disease.

Previous research has focused on the survival of first degree relatives and spouses of long-lived persons. Siblings of centenarians and siblings of nonagenarian descendants had a life-long sustained survival advantage compared to sex and birth cohort matched controls[9,10,16]. In addition, siblings, parents, and offspring of nonagenarian siblings lived significantly longer than members of comparable birth cohorts[11]. Multigenerational studies into the sex-specific inheritance pattern of lifespan and longevity showed inconsistent results however, with either paternal or maternal transmission patterns ([17–33], as reviewed in [34]). Despite the generally observed survival advantage of first degree relatives of longevous subjects, observations on the survival of their spouses and on longevity inheritance patterns remain inconclusive[11,35,36].

The limitations in current inheritance pattern studies are twofold. First, secular trends, such as the increase of life expectancy over time, are not taken into account. Second, parent-offspring analysis usually focuses on a single child per family, thereby omitting the potential of a complete sibship per family[37]. Furthermore, studies have selected long-lived persons based on different criteria, focusing either on multiple siblings or singletons[9–11,16]. It remains to be elucidated whether the stringency of long-lived case selection based on the presence or absence of a long-lived sibling provides a survival advantage in the selected persons compared to birth cohort and sex matched long-lived singletons. Apart from this, research into the survival of first degree relatives and spouses of long-lived persons often struggles to obtain an accurate population based control group, sometimes leading to the generalization of a single birth year control group to other birth years[16]. It is also difficult to compare the survival of parents of long-lived persons to population based sex and birth cohort matched controls because representative cohort lifetables preceding 1900 are often unavailable, except for the Netherlands and Sweden[38]. Overall, research is still inconclusive about the following issues: sex-specific inheritance pattern of longevity, the survival advantage of long-lived sibships as compared to long-lived singletons and about the question whether their parents already had a life-long sustained survival advantage.

To investigate these three issues, we used the data available in the Leiden Longevity Study (LLS). The LLS currently contains 421 complete 3 generational families, which we denote with filial 0 until 2 (F0 – F2). First, we grouped complete F1 sibships to their parental longevity. We defined parental longevity as belonging to the top 1% of their birth cohort[34,39] and constructed four parental groups: Group 1: both parents were long-lived (n=1); group 2: mother long-lived and father not

long-lived (n=17); group 3: father long-lived and mother not long-lived (n=21); group 4: both parents were not long-lived (n=371). We subsequently compared the longevity Family Scores (LFS) of the different groups. Next, we investigated whether longevous siblings had a survival advantage over sex and birth cohort matched singletons using standardized mortality ratios (SMR). We compared the survival of spouses of longevous siblings to sex and birth cohort matched controls. Finally, we estimated lifetime standardized mortality ratios (L-SMRs) to determine if parents of longevous siblings had a life-long sustained survival advantage.

Methods

Leiden Longevity Study

The LLS was initiated in 2002 to study genetic determinants of human longevity. The LLS consists of 421 families and covers 2 generations of living subjects (F1 and F2) who were born between 1864 and 2017. Inclusion took place from 2002 until 2006. Men and women could participate if they were alive and aged >=89 and >= 91 respectively. Both men and women were recruited to have a living sibling meeting the same criteria. Furthermore, the parents of the F1 participants had to be of Dutch Caucasian origin, and the siblings in one family had to descend from the same parents. The sex specific age inclusion criteria represented individuals equal to, or beyond the oldest 0.5 percent of the Dutch population in 2001. There were no selection criteria on health or demographic characteristics. In total 944 longevous F1 participants, who provided blood for research purposes, were included in the LLS (F1). In addition, their offspring and the spouses of their offspring were included (F2).

Relevant for the current study is that genealogical information was collected for the siblings (F1; N=2302), parents (F0; N=842) and spouses (F1; N=809) of the longevous F1 participants (henceforth referred to as siblings, parents, spouses, and participants). All genealogical information was verified by birth or marriage certificates and passports whenever possible. Additionally, verification took place via personal cards which were obtained from the Dutch Central Bureau of Genealogy in the Hague. In 2017 we updated the ages at death and last observation via the currently centralized municipal personal records database. For this study we used two generations (F0 and F1) consisting of 4807 individuals in all 421 families *(Figure 1 and Table 1)* because 86% from the third generation (F2) were still alive.

Lifetables

In the Netherlands, population based cohort lifetables are available from 1850 until 2017[40,41]. These lifetables contain, for each birth year and sex, an estimate of the hazard of dying between ages x and x + n (h_x) based on yearly intervals (n=1) up to 99 years of age. Conditional cumulative hazards (H_x) and survival probabilities (S_x) can be derived using these hazards. In turn, we can determine to which sex and birth year based survival percentile each person of our study belonged to. For example: person "A" was born in 1876, was a female, and died at age 92. According to the lifetable information this person belonged to the top three percent survivors of her birth cohort, meaning that only three percent of the women born in 1876 reached a higher age than person A. We used the lifetables to calculate the birth cohort and sex specific survival percentiles for each individual in the LLS. *Figure A1* shows the ages at death corresponding to the top 10, 5, and 1 percent survivors of their birth cohorts for the period 1850-1960.

Statistical analyses

Statistical analyses were conducted using R statistics version 3.3.0[42].

Standardized mortality Ratio's

To indicate excess mortality or excess survival of groups in the LLS compared to a reference population we used Standardized Mortality Ratios (SMRs). An SMR is estimated by dividing the observed number of deaths by the expected number of

deaths. The expected number of deaths are given by the sum of all individual cumulative hazards based on the birth cohort and sex specific lifetables of the Dutch population. An SMR between 1 and 0 indicates excess survival, an SMR of 1 indicates that the study population shows a similar survival to the reference population, and an SMR above 1 indicates excess mortality. The SMR can be estimated conditional on the specific age at which an individual starts to be observed in the study. This was necessary to avoid selection bias if individuals in a study population were not at risk of dying before a specific age of entry.

$$SMR = \frac{observed\ number\ of\ deaths}{expected\ number\ of\ deaths} = \frac{\sum_{i=1}^{N} d_i}{\sum_{i=1}^{N} H_{t0i}(t_i|t_{0i})}$$

d_i=dead status (1=dead, 0=alive), H_{t0i}=sex and birth year specific cumulative hazard based on lifetable, t_i=timing, referring to age at death or last observation, t_{0i}=liftable age conditioning, in this case from birth (t_{0i}=0), N= group sample size

SMRs were estimated for all first degree relatives (F0 and F1) of the LLS participants (F1) to investigate their survival compared to the Dutch population. Direct or indirect selection effects were taken into account when estimating the SMR by conditioning the lifetable hazards to the age at first death of a specific group. SMRs were also estimated for participants by conditioning to age of inclusion, which varies between 89 and 102 years (see *Table A1* for an overview of conditioning criteria). Note that the lifetables do not contain yearly interval information beyond the age of 99. For this reason the SMR estimations were truncated at 99 years.

To estimate the SMR at every possible starting age we restricted age at death or last observation at yearly thresholds between 0 – 99 years for every group in the LLS, except for the participants because they were selected to have survived >=89/91 years (men/women). We will refer to these age conditioned SMRs as L-SMRs. These L-SMRs provided insight into the specific moment the first degree relatives and spouses had a survival advantage during their lifespan. SMR and L-SMR confidence intervals were estimated using 95% family based bootstrap confidence intervals with 500 resampling cycles to correct for familial dependencies in the LLS data.

Longevity Family Score

To summarize the survival of a specific study population or subsample on the level of families we constructed a Longevity Family Score (LFS). The LFS is related to the SMR, but it is estimated by subtracting the sex, birth cohort, and age conditioned specific cumulative hazards by event status (1 if death and 0 if alive) for each individual in the study population. In a next step, the family mean is calculated which adjusts for family size and results in the LFS. The LFS is related to the Family Mortality History Score described by Rozing et al.[43] and the est(SE) described by Sebastiani et al.[44]. The LFS ranges between -1 and infinity. A score of 0 indicates that the familial longevity resembles that of the normal Dutch population. A score above 0 indicates excess survival and below 0 indicates excess mortality. For example: family "A" scores an LFS of 1. This indicates that we observe 1 death less than expected based on the Dutch population.

$$LFS_i = \frac{expected\ deaths - observed\ deaths}{sibship\ size} = \frac{\sum_{j=1}^{N_i}(H_{t0ij}(t_{ij}|t_{0ij}) - d_{ij})}{N_i}$$

d_{ij}=dead status (1=dead, 0=alive) of individual j, H_{t0ij}=sex and birth year specific cumulative hazard based on lifetable, t^{ij}=timing, referring to age at death or last observation, t_{0ij}=liftable age conditioning, in this case from birth (t_{0ij}=0), N_i=sibship size

To identify the presence of a sex specific inheritance pattern, four groups of F1 sibships (participants+siblings) were constructed according to their parental longevity. We defined parental longevity as belonging to the top 1% of their birth cohort. Group 1: both parents were long-lived (n=1); group 2: mother long-lived and father not long-lived (n=17); group 3: father long-lived and mother not long-lived (n=21); group 4: both parents were not long-lived (n=371). Group 1 was omitted from the analyses because the size was too small and 12 sibships could not be grouped due to missing ages at death of their parents. The LFS was used to summarize F1 sibship survival relative to the parental groups. F1 LFS differences between the groups were tested using the non-parametric Mann-Whitney U test and corresponding 95% exact confidence intervals were reported[45].

Results

To investigate sex specific inheritance and the presence of a life-long sustained survival advantage in the LLS we used two generations covering longevous participants (F1; N= 944), their parents (F0; N= 842), siblings (F1; N= 2302), and spouses (F1; N=809) *(Figure 1).*

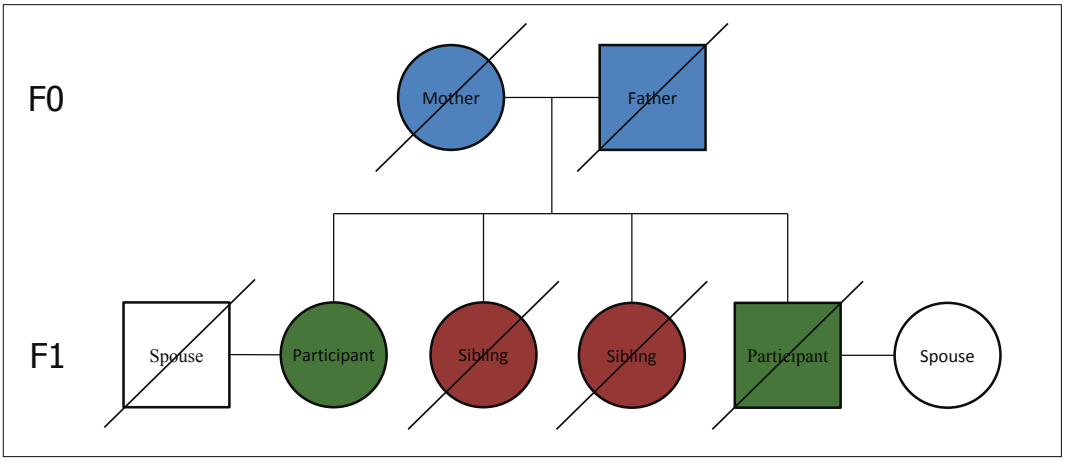

Figure 1: Pedigree map of an example LLS family illustrating the LLS study design.

Circles represent women and squares represent men. Diagonal lines indicate that an individual is deceased. This figure indicates that some participants and their spouses are still alive as of the data of submission. *Table 1* provides an elaborate overview of the LLS data. Colors indicate as follows: BLUE: parental generation (F0); GREEN: participants (F1); RED: siblings (F1); TRANSPARENT: spouses (F1).

The participants were born between 1900 and 1916, and 63% were female (n=595). The participants' mean age at death or at last observation was 97 years and 22 (2%) participants are currently alive. The parents were born between 1850 and 1894 and they are all passed away with a mean age at death of 77 years. We were unable to retrieve the age at death of 22 parents (3%). The siblings were born between 1875 and 1941 and 47% were female (n=1082). The siblings mean age at death was 69 years and the median age at death was 80 years. 365 (16%) siblings are currently still alive while we were unable to retrieve any information on the age at death for 33 (2%) siblings. The mean sibship size for F1 (participants+siblings) was 7.71 (SD=3.4) with a minimum of 2 and a maximum of 17 siblings. The spouses were born between 1882 and 1950. 40% of the spouses were female (n=324) and their mean age at death was 75 years. 27 (3%) spouses are currently alive and for 119 (15%) spouses no age at death or last observation was available *(Table 1).*

Table 1: Leiden Longevity Study sample for participants and first degree relatives

	Parents F0	Participants* F1	Siblings** F1	Spouses F1
Number, N	842	944	2302	809
Deceased, N (%)	820 (97)	922 (98)	1904 (83)	663 (82)
Alive, N (%)	0 (0)	22 (2)	365 (16)	27 (3)
Female, N (%)	421 (50)	595 (63)	1082 (47)	324 (40)
Range birth cohorts	1850-1894	1900-1916	1875-1941	1882-1950
Mean age, years (SD)	77 (14.2)	97 (3.6)	69 (28.3)	75 (14.5)
Median age, years (MAD)	80 (13.3)	97 (4.0)	80 (12.8)	78 (11.0)
Missing age, N (%)	22 (3)	0 (0)	33 (2)	119 (15)

*Participants are enrolled as siblings meeting the age criteria of 89 (men) or 91 years (women). *Siblings are the siblings of participants who did not meet the age criteria yet or who had already been deceased at the time of enrolment. Age refers to either age at death or age at last observation. Missing age means that we have no observation at all. -SD = standard deviation, MAD = median absolute deviation.

LLS data is of high quality

We verified the observations as described by Schoenmaker et al. (2006) based on the first 100 LLS families by estimating SMRs for parents, spouses, and siblings of the complete enrolled LLS *(Table 2)*.

Table 2: Sex specific standardized mortality ratios for 1th degree relatives and spouses of LLS participants

	Sample size	Observed deaths	Expected deaths	SMR (95% CI)
Generation 0 (F0)				
Parents of participants	842	820	1190	0.688 (0.651 – 0.727)
Generation 1 (F1)				
Siblings of participants	2302	1867	2816	0.663 (0.634 – 0.695)
Spouses of participants	809	663	648	1.022 (0.966 – 1.093)

Confidence intervals have been estimated using bootstrapping with 500 cycles. The Dutch life tables do not contain yearly interval information beyond the age of 99. For this reason the SMR calculations have been truncated at 99 years in order to correctly estimate group specific SMR's. No significant differences between men and women have been observed for any category. Observed deaths have been counted after the age of the first death in a group for "parents of participants", "siblings of participants", and "spouses of participants". For the participants observed deaths have been counted after the age of inclusion for each individual separately. This is to correct for selection effects in the data. In line with the counting of the observed deaths, the Dutch lifetables have been age conditioned to match the counting of deaths in the different groups. Equal to the counting of observed deaths, the age conditioning of the lifetables was done to correct for selection effects.

We estimated an SMR of 0.688 (95% CI=0.651–0.727) for parents, indicating that we observed 31.2 percent less deaths than would have been expected based on single individuals from a similar birth cohort and sex. The SMR for siblings was 0.662 (95% CI=0.634–0.695), indicating that we observed 33.8 percent less deaths than would have been expected based on single individuals from a similar birth cohort and sex. Spouses had an estimated SMR of 1.022 (95% CI=0.966–1.093). This indicates that we have not found differences between the survival of spouses and single individuals from similar birth cohorts and sex.

Maternal transmission of longevity

To determine an inheritance pattern based on information of not just single individuals but an entire sibship we used a Longevity Family Score (LFS) to summarize sibship survival. We grouped sibships (F1, participants+siblings) according to their parental (F0) longevity (parental longevity was defined as belonging to the top 1% survivors of their birth cohort) and compared the median group LFS of the complete sibships. *Figure 2* shows that all F1 sibship groups, on average, had an excess survival as compared to single individuals from the same birth cohorts and sex, as indicated by the median scores which were all above 0. Sibships with a long-lived (LL) father and a non-long-lived (NL) mother had 1.21 (median LFS) less observed deaths in reference to the Dutch population and a mean sibship size of 8.34 (SD=3.4). Sibships with an LL mother and an NL father had 1.62 (median LFS) less observed deaths with a mean sibship size of 5 (SD=1.9) and sibships with both parents NL had 1.1 less observed deaths with a mean sibship size of 7.95 (SD=3.4). As a result, sibships with long-lived mothers and non-long-lived fathers showed larger LFSs than sibships with long-lived fathers and non-long-lived mothers (median difference in LFS of 0.41; 95% CI=0.07–0.77 ; P=0.024) Similarly, they showed larger LFSs than sibships with both parents non-long lived (median difference in LFS=0.48; 95% CI=0.15–0.79 ; P=0.008). We did not observe differential survival between sons and daughters with a long-lived mother *(Figure A2).* In conclusion, we observed a maternal transmission pattern of human longevity with no evidence of a differential survival advantage for sons and daughters.

Last life-phase survival advantage of siblings over singletons

To test if longevous F1 participants had a survival advantage over birth cohort, sex and inclusion age matched singletons we estimated sex-specific SMRs for the participants *(Figure 3A).* An SMR of 0.814 (95% CI=0.757–0.884) was estimated for the participants, indicating that as a group the participants had 18.6% less deaths than expected based on single individuals from similar birth cohorts and sex. Female participants had a slightly larger survival advantage (0.804 (95% CI=0.738–0.894)) than male participants (0.828 (95% CI=0.742–0.943)) although this difference was not significant.

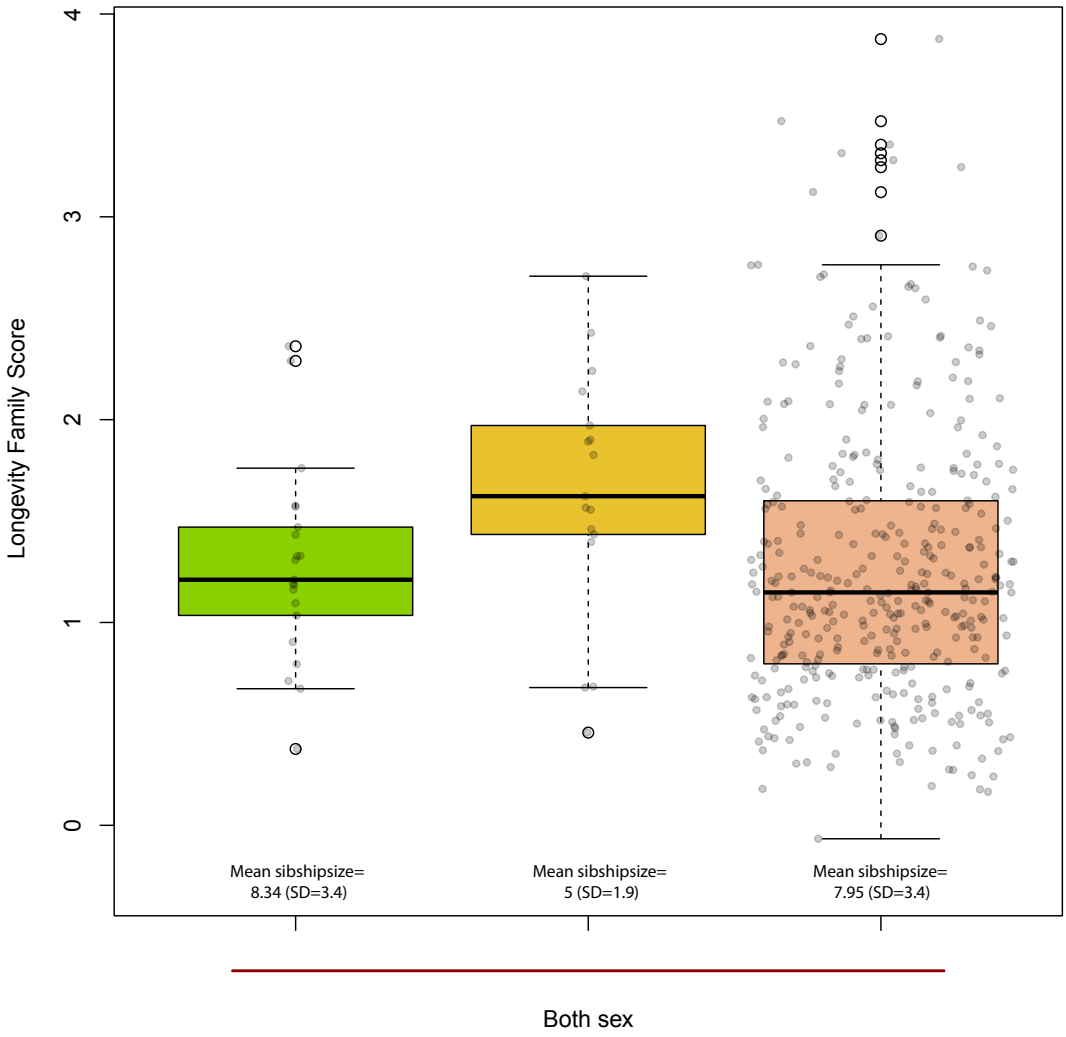

Figure 2: Median Longevity family score per sibship with one or none long-lived parent.

Each gray dot represents a complete sibship. Green boxplot represents the group of sibships with long-lived father and a non-long-lived mother (N_sibships=21 ; N_individuals=177). Orange boxplot represents the group of sibships with a long-lived mother and a non-long-lived father (N_sibships=17 ; N=individuals=85). Light brown boxplot represents the group of sibships with both parents not long-lived (N_sibships=371 ; N_individuals=2949).

Life-long sustained survival advantage of siblings and parents but not for spouses

Whether first degree relatives and spouses of the participants had a survival advantage over their entire lifetime was studied by estimating L-SMRs. *Figure 3B* shows that siblings had a significant survival advantage compared to individuals from similar birth cohorts and sex at any point of their lifetime distribution until the threshold of 97 years, although the SMR at 98 years was again significant. The mean L-SMR was 0.680 and the median L-SMR was 0.660. No sex differences were identified at any age threshold. We observed that spouses had a non-significant L-SMR until age 74, indicating that they were similar to sex and birth cohort matched individuals from the general population. Beyond age 74 there was a small but significant survival disadvantage (min SMR=1.09 and max SMR=1.32) and from age 91 until 94 the effects were not statistically significant anymore. Among spouses, no statistically significant differences between husbands and wives could be detected at any age threshold. The mean L-SMR was 1.050 and the median L-SMR over all age points was 1.030 *(Figure 3C)*. Finally, we were able to study the life-long survival for parents of longevous participants *(Figure 3D)*. Parents had a significant survival advantage compared to individuals from the same birth cohort and sex at any point of the parents' lifetime distribution until 93 years. After 93 years the SMR estimates were still below 1 although not statistically significant, probably due to small sample size. The parental mean and median L-SMR were 0.510 and 0.688 respectively. No sex differences were identified at any age threshold. Exact values corresponding to *Figure 3* can be found in *Table A2*.

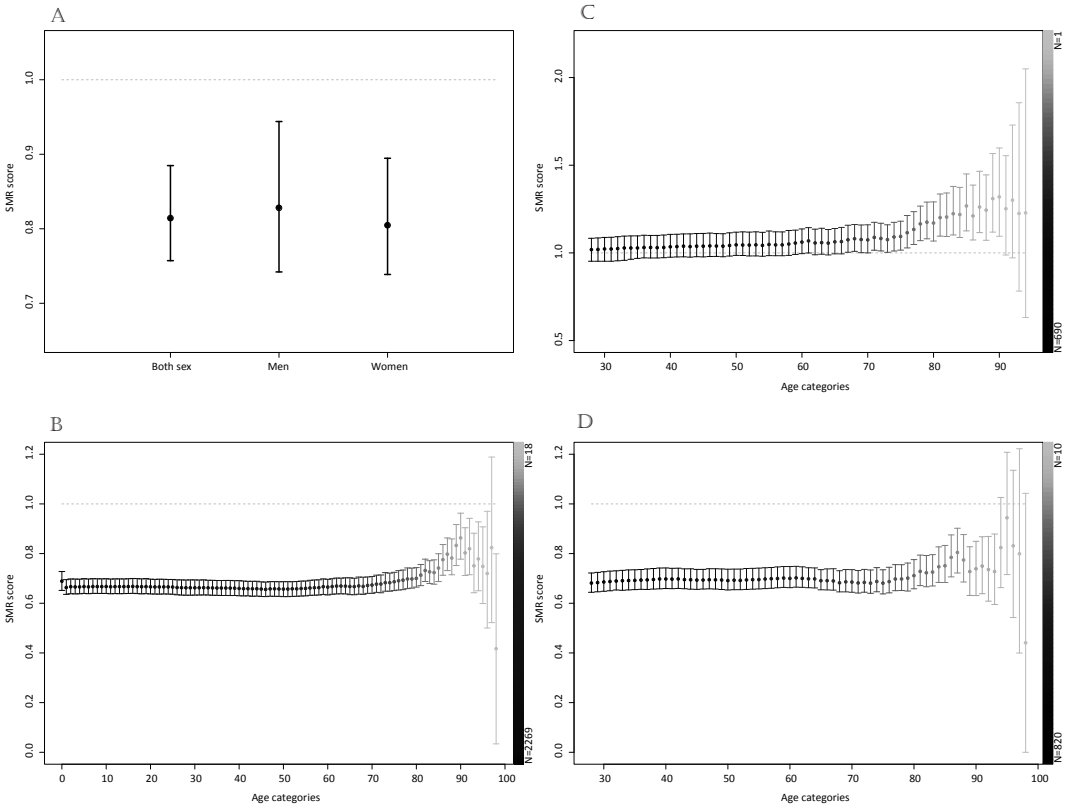

Figure 3: Standardized mortality ratio (SMR) for participants and lifetime SMR for first-degree relatives + spouses

(A) SMR for the LLS participants, (B) all age SMR for sibs (F1) of participants, (C) all age SMR for spouses (F1) of participants, and (D) all age SMR for parents (F0) of participants. The horizontal dotted line illustrates the SMR threshold value of 1. The nodes are SMR point estimates. The error bars represent the family bootstrapped confidence intervals. The colors in (B), (C), and (D) illustrate the sample size at every cutoff. The higher the age threshold, the lower the sample size, and hence, the lighter the color. The bars at the right side of the subfigures show the sample size associated with the colors of the SMRs.

Discussion

We investigated the survival of the longevous F1 LLS participants (who are longevous siblings) selected in the Leiden Longevity Study, and their F1 siblings, F0 parents, and F1 spouses. Based on the lifespan data of entire sibships (F1, participants+siblings), we observed a maternal transmission pattern of longevity with equal probability to sons and daughters. As compared to inclusion age matched singletons from similar birth cohorts and sex, LLS participants had 18.6% less observed deaths than expected, and thus a survival advantage. In the LLS the spouses of the participants had a life-long sustained survival pattern similar to the general population. Finally, we conclude that parents and siblings of the LLS participants had a life-long sustained survival advantage as compared to individuals matched on birth cohorts and sex.

Family longevity scores (FLS) were used to explore whether human longevity was transmitted preferentially through the maternal or paternal line, using the entire sibship information instead of only that of one single child per family. All sibships had an increased survival compared to individuals from the same birth cohort and sex, regardless of their parental longevity, because we selected LLS participants to have lived >= 89 and 91 years for men and women respectively. However, the median FLS for sibships with a long-lived mother and a non-long-lived father was 0.41 (P=0.024) higher than for sibships with a long-lived father and a non-long-lived mother, and 0.48 (P=0.008) higher than for sibships with both parents non-long-lived. This indicates that in the LLS longevity was transmitted preferentially via the maternal line. This maternal transmission of longevity is in concordance with the mitochondrial transmission hypothesis which posits that longevity may be transmitted through mitochondrial DNA from mothers to her offspring[8]. Though, this theory argues that because mitochondria are only maternally inherited they are under selection pressure for optimized compatibility with only the female genome, we have no evidence that there is preferential transmission of longevity from mothers to daughters. Another explanation connects to Fogel's (1997) theory of technophysio evolution which explains that in the turn of the 19th to the 20th, century childhood and early life mortality decreased significantly. This decrease was attributed to an increased birth weight and height of children and young adults respectively[46]. Since mothers are pivotal in this process it might be that the long-lived mothers were able to give birth to such healthy children whereas this may not have been the case for non-long-lived mothers, irrespective of the beneficial effect that 19th century long-lived fathers may have provided. The similarity in LFS for sibships with a long-lived father and a non-long-lived mother (LFS=1.21) and sibships with both parents non-long-lived (LFS=1.14) indicates the small influence of paternal effects compared to maternal effects. This absence may indicate that paternal socio-economic status in the LLS is of marginal influence to the intergenerational transmission of longevity[47,48]. Sibships with a long-lived mother and a non-long-lived father had not only had a higher LFS, they also had a mean sibship size of 5 whereas the two other categories had a mean sibship size of 8.34 and 7.95. In general, the probability of finding long lived subjects in families increases with sibship size[49]. The finding of longevity among children in small sibships (with a long-lived mother) may therefore indicate that the longevity is less likely to be prominent by chance. The smaller sibship size of LL mothers may be explained by a trade off in longevity families, either based on environmental (i.e. limited economic resources) or biological (i.e, reproductive capacity) factors. The discordant parental groups were quite small *(Figure 2)*. We identified sibships with a long lived father but not mother, and vice versa (N_sibships=21 ; N_individuals=177 and N_sibships=17 ; N=individuals=85) which interestingly shows that the maternal transmission effects are found not in

all, but in a subset of LLS families.

To investigate familial clustering of longevity, studies selected long-lived subjects based on multiple siblings or singletons[9–11,16]. So far it was unclear whether a sibling based selection provides a survival advantage over singletons. We showed that longevous siblings (F1 LLS participants) indeed had an 18.6% survival advantage over inclusion age, birth cohort, and sex matched longevous singletons. The effect can be considered large because the observational period focuses on the last stage of life (age >= 89 and 91 for men and women), especially when taking into account that siblings of LLS participants, who's full life course was observed showed a 33.7% survival advantage. It might even be expected that confining the sample to participants consisting of 3 or more longevous siblings increases the survival advantage. We did not, however, have the sample size to stratify our analyses to specific numbers of longevous participants within a family. Furthermore, we accounted for direct selection effects, although we could not directly account for the possibility that more healthy persons enrolled in the LLS than unhealthy persons or vice versa. We, however, did not expect that this has influenced our results since the first participants died only a few weeks after inclusion. We conclude that, when compiling a long-lived study cohort, selecting longevous siblings is a more stringent selection than longevous singletons of the same age.

Literature is inconclusive about the potential survival advantage of spouses of long-lived persons[10,11,35,36]. We showed, in a large group, that spouses of longevous LLS participants (N=809) had an equal survival to the general population until the age of 74. Beyond 74 years we observed a small excess mortality. We have no other explanation for this finding than the fact that this excess mortality beyond 74 years may be a function of small sample size. Pedersen et al. (2017) observed a survival advantage in the long life family study for spouses of long-lived siblings when comparing them to a birth cohort and sex matched control group. The authors point to assortative mating as a factor explaining the survival advantage for spouses of longevous participants[10]. An earlier Quebec study also reported a survival advantage of spouses[35] and a study of Southern Italy found male nonagenarians to outlive their spouses, whereas this was not the case for female nonagenarians[36]. Clearly, biological, environmental, and cultural factors influence survival to advanced ages in longevous families.

Because of unique Dutch lifetables dating back to 1850, we were able to show that parents of longevous LLS participants had a life-long sustained survival advantage compared to birth cohort and sex matched controls, until at least the age of 93 years. Beyond 94 years the confidence intervals increased due to a limited sample size. The life-long sustained survival advantage of first-degree relatives indicates a familial clustering of human longevity, which may be the result of the absence of deleterious genetic mutations[50,51] or the presence of genetic mutations protecting from aging related diseases[52]. Genetic studies aimed at identifying longevity loci promoting a life-long survival advantage up to the highest ages requires a focus on extreme individuals: cases belonging to the top 1-5% survivors with comparable parents. Recent genetic studies in the large UK Biobank[50,51] focused on subjects of 70 years on average without a parental selection[51] or selecting on parents belonging to the top 10% survivors[50]. This selection resulted in loci known to influence healthy ageing and mortality in middle and older age rather than exceptional longevity. As alternative to genetic influences, shared lifestyle or environmental factors may influence the longevity clustering in families. With the SMR analyses we could not adjust for environmental and lifestyle factors. However, the fact that we found spouses to survive comparable to the general population and that first

degree relatives (siblings and parents) had a life-long sustained survival advantage suggests a familial/genetic influence on human longevity, possibly acting from early life onward.

Longevity clusters within specific families and insight into this familial clustering is important in gaining knowledge of factors involved in a life-long survival advantage up to the highest ages. Knowledge about the inheritance pattern of longevity may be useful for genetic studies trying to discover longevity related genes. For example, effects of mitochondrial genes on human longevity should be investigated in those families with a history of maternal transmission of human longevity. Furthermore, research aiming to establish a study cohort of long-lived persons should ideally take family information into account, because we have demonstrated an enhanced survival for longevous siblings (LLS participants) over birth cohort and sex matched singletons. In the LLS, spouses seem comparable to the general population, making them a suitable comparison group for various health-related phenotypes as well as longevity. Lastly, as compared to sex and birth cohort matched individuals, parents of the LLS participants at the turn of the 19th century have a life-long sustained survival advantage up to the highest ages which was previously reported for the 20th century survival of siblings of longevous singletons[9,10,16]. This indicates that when studying the determinants of longevity factors involving the entire lifespan may contribute and emphasize the importance of longitudinal population based studies in the search for protective factor for age-related disease.

Conflict of interest

The authors declare that they have no conflict of interest

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Informed consent

Informed consent was obtained from all Leiden Longevity Study participants

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