

Siblings of Okinawan Centenarians Share Lifelong Mortality Advantages

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Okinawa, an isolated island prefecture of Japan, has among the highest prevalence of exceptionally long-lived individuals in the world; therefore, we hypothesized that, within this population, genes that confer a familial survival advantage might have clustered. We analyzed the pedigrees of 348 centenarian families with 1142 siblings and compared sibling survival with that of the 1890 Okinawan general population cohort. Both male and female centenarian siblings experienced approximately half the mortality of their birth cohort–matched counterparts. This mortality advantage was sustained and did not diminish with age in contrast to many environmentally based mortality gradients, such as education and income. Cumulative survival advantages for this centenarian sibling cohort increased over the life span such that female centenarian siblings had a 2.58-fold likelihood, and male siblings a 5.43-fold likelihood, versus their birth cohorts, of reaching the age of 90 years. These data support a significant familial component to exceptional human longevity.

THE familial component to human longevity, including whether there exist genes that enhance the ability to achieve exceptional old age (hereafter referred to as “longevity genes”), is a fundamental issue in modern biogerontology (1–3). This issue generates an ongoing and vigorous debate among evolutionary biologists, geneticists, demographers, and others in the field of aging.

Some researchers have suggested that the evolutionary biology theory presupposes no advantage to select for genes that would allow an organism to live past its reproductive years, and thus the idea of longevity genes is fundamentally flawed (4,5). This viewpoint would preclude evolutionary selection for genes that confer longevity but does not preclude clustering of genes in populations that may modulate longevity under the right circumstances. For example, genes might be selected for that could extend an organism’s life during times of scarcity to ensure reproductive success. This infers the existence of genes that might confer longevity through reducing risk for early-life mortality.

Others suggest that there are clear advantages to having members of the species live well beyond reproductive years, to help rear the young for example, as argued in the Grandmother hypothesis (6). This philosophy suggests an evolutionary reason for late-acting genes that impart a survival advantage through reducing late-life mortality.

Numerous studies also suggest that survival to very old age has both strong environmental components and strong genetic components—and remarkable plasticity (1,2,7,8). For example, animal data show that life span in rodents can be increased by up to 70% through restricting energy intake

(9,10). Genetic studies have also shown that single-gene mutation models of mammals (e.g., mice) may have life-span extension of up to 70% (3,10,11). Other species, from yeast to earthworms, show even greater life-span increases under these two paradigms (3,9,10). Thus environmental factors (such as diet) appear important to longevity, but genetic factors also modulate longevity. However, which particular genes and how differential expression of these genes may influence mortality at different times over the life course is not known.

Recent work suggests that gene expression profiles linked to exceptional longevity may be inducible to a similar degree by caloric restriction in both young and old animals approximately doubling remaining life span in both (8). In addition, older animals, although possessing lower baseline levels of certain age-related gene products, appear as capable as younger animals of expressing these gene products when required for growth and repair (12). This capability implies a remarkable plasticity throughout the life span to modulate the expression of genes that affect growth and repair processes linked to aging. Possession of favorable gene polymorphisms might result in better repair of damage linked to senescence and, ultimately, slower age-related decline.

Human studies of dietary, psychological, and socio-behavioral factors also demonstrate some impact on healthy aging and survival, but their relationship to exceptional longevity or to the limits of life span are less clear (2,13,14). Studies of human gene single nucleotide polymorphisms (SNPs) have demonstrated significant associations with longevity. Genetic variants that modulate insulin/insulin-like growth factor-1 (IGF-1) signaling (15), inflammation (16–18),

and stress resistance (19), among other factors, differ significantly between very long-lived humans (such as centenarians) and shorter lived humans. However, the temporal expression of these genes is not known.

Several studies have attempted to quantify the heritable component of human longevity. Reported heritability of human longevity ranges from negligible to 50% (3,20–22). This heritability factor has most often been studied in populations of humans who live to an average age but not in exceptionally long-lived individuals, and thus would tend to underestimate the genetic contribution to longevity in long-lived families (23). Familial patterns of longevity in humans provide an important clue to the identification of genes that modulate the aging process. Family linkage studies have been important in the discovery of heritable traits and are an important potential tool for discovering the heritable aspect of human longevity (24). For example, studies of U.S. centenarian pedigrees and Mormon pedigrees support a familial component to longevity (25,26).

To successfully design family-based linkage studies that might identify longevity genes, there must be a high probability that long-lived probands will have family members who will also possess these genes. We have shown in a previous extensive study of centenarian pedigrees and exceptional human longevity that centenarians and other long-lived individuals in Okinawa, Japan, do appear to share familial longevity traits (27). In addition, we have also shown that older Okinawans possess differences in Human Leukocyte Antigen (HLA) allele frequency compared with younger, unrelated controls (16,17). HLA consists of three classes of proteins (I, II, III) that function as genetic fingerprints on white blood cells and platelets. Part of the human Major Histocompatibility Complex (MHC), HLA proteins play a critical role in activating the body's immune system, and may play a role in the immunogenetics of aging (28).

In one of the earliest studies using centenarians as a genetic model for human longevity research, we demonstrated that Okinawans aged 90 years or older had decreased frequency of HLA-DR9 and increased frequency of HLA-DR1 alleles compared with younger controls, among other antigenic differences (16). In addition, heterozygosity for the HLA-DR allele was decreased in Okinawan centenarians. In a subsequent study, molecular typing of 129 Okinawan centenarians and 129 adult controls also supported a high frequency of the HLA-DR1 allele in Okinawan centenarians (17). HLA DR1 is associated with a strong response to mitogens and some infectious antigens (16), and antigenic differences may affect the strength, effectiveness, and duration of local and systemic immunoinflammatory responses, which may in turn impact longevity.

Although there appears to be a significant familial component to human longevity, with the exception of the study by Perls and colleagues (21), there does not appear to be a thorough examination of the question of mortality risk across the life span in very long-lived individuals and their family members. This makes it difficult to assess whether early- or late-acting genes might confer a survival advantage. Another limitation of previous studies is that they have been conducted in genetically and socially heterogeneous popula-

tions, with different ethnicities, levels of education, income, or other factors that may confer a survival advantage. These factors have made it exceedingly difficult to disentangle genetic from environmental causes of exceptional longevity.

In the current research, we report a population-based study of 348 centenarian families from the genetically and socially homogeneous island population of Okinawa, Japan, where the longest life expectancy (29) and highest prevalence of exceptionally long-lived individuals in Japan has been reported (30). We compare age-specific mortality rates and survivorship in centenarian siblings up to the age of 90 years with their corresponding birth cohort. The population of Okinawa is of significant interest with respect to the study of longevity because they appear to possess among the world's longest survival (29,31) and the highest prevalence of exceptionally aged individuals in Japan, if not the world (7,30).

METHODS

The Okinawa Centenarian Study (OCS) is an ongoing, population-based study that began in 1976 of 100-year-olds and other selected elderly persons aged 65 years or older residing in the Japanese prefecture of Okinawa (32). Data are collected by visit to the centenarian's place of residence. At the initial examination, a full geriatric assessment is performed, including physical examination and assessment of activities of daily living. Other data collected include socio-demographic characteristics, medical history, anthropometric measures, diet, smoking status, alcohol consumption, family pedigree, and blood samples. Blood pressure is measured and a 12-lead resting electrocardiogram is recorded. All centenarian ages are confirmed through their *koseki* (household registration) records kept at city, town, or village offices. The details have been reported elsewhere (32).

After receiving Institutional Review Board approval from Okinawa International University, Pacific Health Research Institute, and Kuakini Hospital, the pedigrees of centenarian families for probands were entered in the Cyrillic Pedigree software program (Cyrillic Software, Wallingford, U.K.). This graphical entry method significantly reduces the likelihood of errors. The data were then exported to files formatted for statistical analysis.

All pedigree data were reported by the centenarian probands (if cognitively intact) and confirmed by the next of kin or reported by the next of kin, if the proband was not cognitively intact. There were 348 centenarian pedigrees with age at interview (if alive) or age at death (if dead) for siblings. A total of 1142 siblings, 615 women and 527 men, were available for analysis, all of them of Okinawan ethnicity. The probands had a median of 3 siblings with a range of 1–10. At the time of data collection, 115 (19%) of female siblings and 67 (13%) of male siblings were still alive. All probands were born between 1874 and 1902 with a median birth year of 1890.

For siblings still alive at the time of data analysis, observations were censored. We then compared the centenarian sibling mortality and survivorship to the mortality and survivorship experience of the general Okinawan population from the same birth cohort. All of the centenarian probands

were excluded from the analysis, because the siblings of the centenarians were the focus of this study. Mortality rates for siblings were calculated from tabulations of siblings' age at death and censored observations. Both the death counts and exposure estimates are aggregated in 5-year age groups, and standard demographic methods are used to calculate the mortality rate and its variance. More specifically, the mortality rate for each age interval x is calculated as the following:

$$d_x = D_x/E_x, \quad \text{and} \quad E_x = N_x - \frac{1}{2}(D_x + C_x),$$

where D_x is the number of deaths in the interval, C_x is the censoring in the interval, and N_x is the total number of persons at the start of the interval. The variance of the estimated mortality rate is calculated according to Poisson distribution (33):

$$\text{var}(d_x) = d_x/E_x.$$

The survival rate for interval x is computed as following:

$$p_x = (R_x - D_x)/R_x, \quad \text{and} \quad R_x = N_x - C_x/2.$$

The survival curves, S_x , are computed as $s_x = p_0 p_1 \dots p_{x-1}$. The variance of the estimated survival curve at x is computed as following, based on the binomial distribution and Greenwood's formula (34):

$$\text{var}(s_x | R_1, R_2, \dots, R_x; D_1, D_2, \dots, D_x) = s_x^2 \sum_{i=0}^{x-1} \frac{D_i}{R_i(R_i - D_i)}.$$

The sibling mortality rates and survival curves are compared with the corresponding mortality rates and survival curves of the 1890 birth cohort of the general Okinawan population to determine whether centenarian siblings had lower mortality and higher survival probability versus the general Okinawan population. These data were derived from the Health and Welfare Dataset of the Okinawa Prefectural Government (35), which provides population and health-related data for age- and gender-specific groups for the general Okinawan population. Most of the data are for 5-year age groups and in 5-year intervals. There are no data for certain time periods in and around the Second World War (i.e., 1940–1955). For the purposes of these comparisons, the closest chronological data were used in the computations.

As there was some potential for underreporting of sibling deaths due to recall bias, survival probabilities reported here are conditional on survival to age 20. This minimizes the effect of such errors on cumulative survival probability. More than one family member was interviewed to obtain family pedigree data. Confidence intervals for relative death rates and survival probabilities are the estimated value plus or minus two times the standard error. Data for the 1890 cohort were treated as fixed numbers (with zero variance) when calculating the standard errors used for confidence intervals, which did not result in loss of accuracy.

RESULTS

Mean age at death was higher for centenarian siblings than for the 1890 general birth cohort from Okinawa. Female centenarian siblings attained a mean age at death of

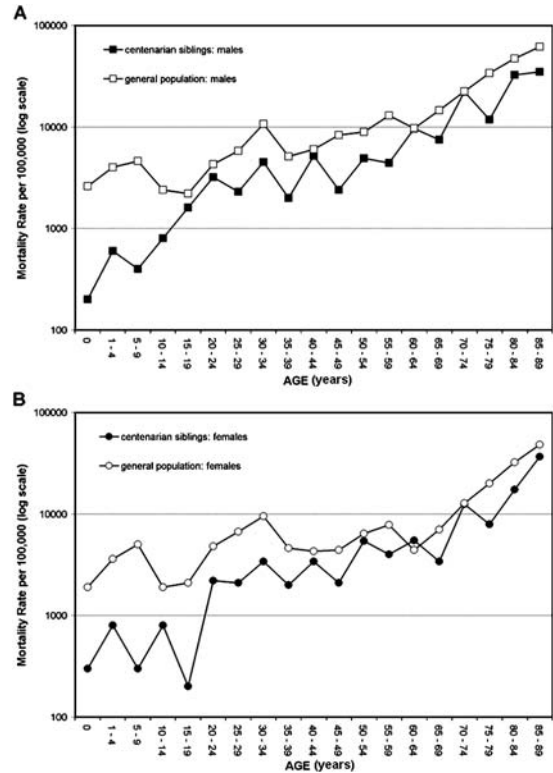


Figure 1. Age-specific mortality rates for centenarian siblings versus 1890 birth cohort, by sex.

68.4 years, and male siblings a mean age at death of 64.0 years. In comparison, mean age at death for birth cohort-matched individuals from the general Okinawan population are estimated at 56.9 years for women and 52.0 years for men, respectively (35).

Age-Specific Mortality Rates for Centenarian Siblings Versus 1890 Birth Cohort, by Sex

Age-specific mortality rates for centenarian siblings, by sex, are illustrated in Figure 1. This figure shows age-specific mortality rates for male and female siblings of centenarians and for the general population of Okinawa prefecture born in 1890. Age-specific mortality is lower in centenarian siblings at almost all ages than in the general Okinawan population. The comparable relative mortality rates, illustrating the lower relative mortality risk of centenarian siblings versus the general Okinawan population from the 1890 birth cohort, can be seen in Table 1. Interestingly, the tail end of the distribution representing infant mortality (<1 year old) slopes downward for both centenarian siblings and the general Okinawan population. This slope is in contrast to the usual J-curve seen in age-specific mortality in most industrialized countries. The J-curve arises because both infant mortality and early adult mortality are higher than child mortality in most industrialized countries. The downward slope seen in the Okinawan data is most likely due to underreporting of infant mortality in both centenarian siblings and the total Okinawan population. Underreporting was a common occurrence at that time in Japan and many other

Table 1. Relative Mortality Rate (MR) With 95% Confidence Intervals (CI) of Centenarian Siblings Versus Okinawa 1890 Birth Cohort

Age Group, y	Women		Men	
	Relative MR	95% CI	Relative MR	95% CI
0	0.18	0.00–0.42	0.07	0.00–0.22
1–4	0.12	0.02–0.22	0.07	0.00–0.17
5–9	0.07	0.00–0.16	0.08	0.00–0.20
10–14	0.43	0.05–0.81	0.32	0.01–0.63
15–19	0.08	0.00–0.23	0.71	0.22–1.20
20–24	0.45	0.21–0.70	0.74	0.38–1.11
25–29	0.31	0.13–0.48	0.39	0.16–0.62
30–34	0.35	0.20–0.51	0.42	0.24–0.59
35–39	0.43	0.18–0.69	0.39	0.13–0.64
40–44	0.79	0.42–1.15	0.87	0.52–1.23
45–49	0.48	0.20–0.77	0.28	0.11–0.46
50–54*	0.83	0.52–1.15	0.55	0.31–0.79
55–59	0.51	0.28–0.74	0.34	0.18–0.50
60–64*	1.25	0.76–1.74	0.99	0.66–1.32
65–69*	0.49	0.24–0.74	0.51	0.31–0.71
70–74*	0.96	0.70–1.23	1.00	0.75–1.24
75–79	0.39	0.25–0.54	0.35	0.22–0.48
80–84	0.54	0.39–0.68	0.69	0.52–0.86
85–89	0.76	0.60–0.93	0.57	0.40–0.74

Note: *These age groups did not have the corresponding data for Okinawa general population. The data from nearest time were used in the analysis.

countries. In studies of older individuals recall bias can be a problem, and there is the potential for underreporting of centenarian sibling mortality due to recall bias of the centenarians or their proxies, especially regarding siblings who died early in life (21). To minimize the effect of such bias, survival probabilities are reported from the age of 20 onward in Figure 2.

Survival Probability of Siblings of Centenarians Versus 1890 Birth Cohort

Figure 2 illustrates survival probabilities for men and women from the age of 20 years for siblings of centenarians compared with the 1890 general population birth cohort. The survival probabilities for the two groups do not differ in a major way during early adulthood, but they begin to diverge from the mid-30s onward and significantly separate in the 50s and 60s. At older ages, where the force of mortality is strongest for populations, we can see that the siblings

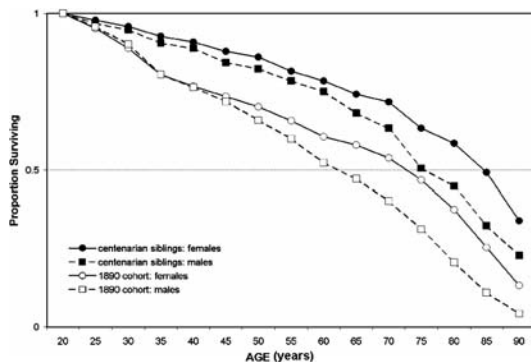


Figure 2. Survival probability of centenarian siblings versus 1890 birth cohort.

Table 2. Cumulative Survival Ratios (CSRs) with 95% Confidence Intervals (CI) for Siblings of Centenarians Versus Okinawan 1890 Birth Cohort

Age, y	Women		Men	
	CSR	95% CI	CSR	95% CI
20	1.00	1.00–1.00	1.00	1.00–1.00
25	1.03	1.01–1.04	1.01	1.00–1.03
30	1.08	1.06–1.10	1.05	1.03–1.07
35	1.15	1.13–1.18	1.13	1.09–1.16
40	1.19	1.16–1.22	1.16	1.13–1.20
45	1.20	1.16–1.23	1.17	1.13–1.21
50	1.23	1.19–1.27	1.25	1.20–1.30
55	1.24	1.19–1.29	1.30	1.25–1.37
60	1.29	1.24–1.35	1.44	1.37–1.51
65	1.28	1.22–1.34	1.45	1.36–1.53
70	1.33	1.26–1.40	1.57	1.47–1.68
75	1.35	1.27–1.43	1.62	1.48–1.76
80	1.56	1.45–1.67	2.17	1.96–2.38
85	1.94	1.78–2.10	2.97	2.59–3.35
90	2.58	2.28–2.87	5.43	4.52–6.32

of the centenarians, both male and female, have maintained a mortality advantage that began largely in adulthood and continues until the very oldest ages.

Cumulative Survival Probability in Siblings of Centenarians Versus 1890 Birth Cohort

Table 2 and Figure 3 illustrate how the relative mortality advantage that exists for centenarian siblings of both sexes across almost every age group results in an increasing survival advantage over the life course. This result does not mean that the mortality advantage seen in centenarian siblings is increasing at older ages. Indeed, as can be seen in Table 1 and Figure 1, this mortality advantage varies widely per 5-year age group but not in a systematic direction, averaging roughly 50% lower mortality for centenarian siblings across age groups. Table 2 and Figure 3 demonstrate that cumulative survival probability increases rapidly in centenarian siblings as they age because the cumulative mortality advantage of the centenarian siblings grows over the course of their lives. We can also see that at a given age, male survival ratios are higher than female survival ratios because a larger fraction of men have died; therefore, the male siblings' cumulative advantage compared to their birth cohort

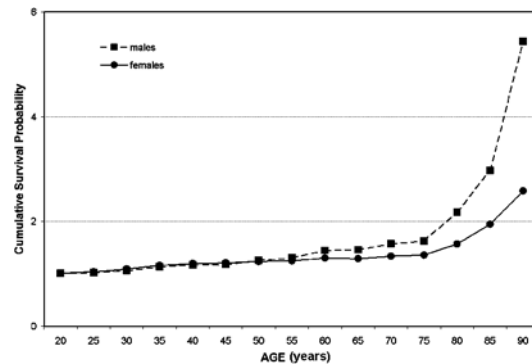


Figure 3. Cumulative survival probability in siblings of centenarians versus 1890 birth cohort.

counterparts is greater than that for women of the same age. Most importantly, compared with the 1890 birth cohort, female siblings were 2.58 times as likely to reach the age of 90, whereas male siblings were 5.43 times as likely.

Percentage of Siblings of Centenarians and Controls Who Reached 90 Years of Age When Controlling for Extrinsic or Unknown Causes of Mortality

To control for potential bias inherent in using total mortality as an indicator of biological causation, deaths that were due to causes that are extrinsic to aging or disease (such as deaths from accidents, war-related deaths, suicides, homicides) were eliminated from the analysis for a subsample of the centenarian sibling population. Excluding accidental deaths from the analysis imparts biological reasoning into what is otherwise a purely mathematical process (36). In such a case, the familial effect of exceptional longevity would be underestimated. Because our centenarian sibling population is much smaller than the total Okinawan population, it is more sensitive to this potential problem than are large populations. This analysis also is important in the case of Okinawa due to the large number of deaths related to the Second World War.

Figure 4 shows a subset of the sibling centenarian population that excluded all families in which a sibling died from an extrinsic cause versus control families from the same village and birth cohort in Okinawa. The analysis performed on this smaller subset was carried out with data collected on siblings from 114 centenarian families and on siblings from 11 cohort-matched control families to deal with the above mentioned problem of controlling for accidents, war-related deaths, and other extrinsic causes of death unrelated to aging or disease processes. The 11 control families were chosen based on the following characteristics: (i) all came from the same towns or villages as the centenarian families; (ii) there were at least three siblings in the family; (iii) the oldest of the siblings would have been a centenarian herself or himself, had he or she still been alive (same birth cohort as the centenarian siblings); and (iv) all siblings who died from deaths due to war-related causes, accidents, suicides; those missing; or those whose ages at death were unknown were eliminated.

Ages at death were calculated for the centenarian siblings and control family siblings. The percentage of siblings who died at age 90 years or older was calculated and compared using the chi-square test (Figure 4). Of the 46 male centenarian siblings and 17 male control family siblings, 24% of the centenarian siblings versus 12% of the control siblings lived to at least 90 years of age ($p = .29$). Of 90 female centenarian siblings and 22 female control siblings, 49% versus 14%, respectively, lived to at least 90 years of age ($p = .003$). When male and female centenarian siblings were combined and compared with control siblings, 40% of centenarian versus 13% of controls lived to at least 90 years of age ($p = .001$). The small number of male centenarian siblings limited the power to detect a significant difference versus controls. However, when sufficient numbers of individuals were available, as with female centenarian siblings (and total centenarian siblings), there were highly significant survival advantages to age 90 years compared with controls. Thus,

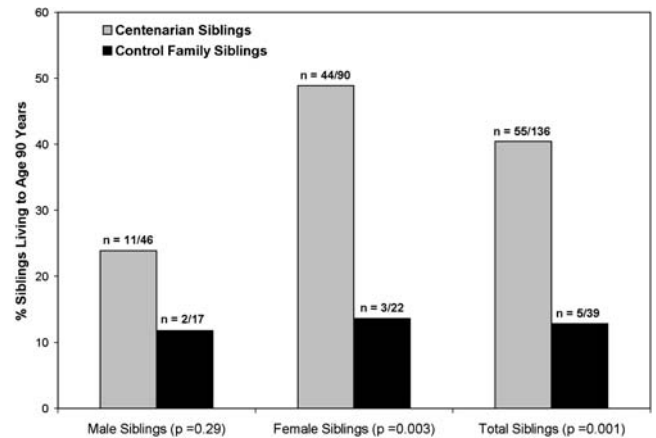


Figure 4. Percentage of siblings of centenarians and controls who reached age 90 years, when controlling for extrinsic or unknown causes of mortality.

siblings of centenarians appear more likely to reach the age of 90 years when compared to birth cohort-matched control families from the general population, after controlling for extrinsic causes of mortality.

DISCUSSION

Okinawans are well known for harboring a very low all-cause mortality rate, and possess the longest life expectancy at older age groups in Japan (29), the world's longest lived country (31). Consequently, Okinawa has accumulated a very high prevalence of elderly individuals including Japan's highest prevalence of centenarians, at 51.4 centenarians per 100,000 persons versus approximately 20 centenarians per 100,000 persons in Japan overall (29) and approximately 10–20 per 100,000 persons in most Western countries (37,38). The high centenarian prevalence exists despite Okinawa possessing the highest birth rate in Japan and the emigration of large numbers of the current centenarian cohort in the early 1900s. There is evidence that much of the low mortality at older ages is due to environmental factors such as healthy diets, physical activity, social factors, and medical care (39–43). This may account, in part, for the exceptional longevity of its inhabitants.

However, as an isolated, island population with restricted gene flow, there is potential for clustering of genes that may impact human health and survival (44), as may be seen in the island populations of Iceland (45,46) and Sardinia (47). Humans most likely to possess genes that impart longevity advantages are those who have survived demographic and selective forces, such as centenarians.

The sibling design of the present study was meant to address the familiarity or “correlation within families” of human longevity. In traditional genetics, assessing familiarity precedes the division of traits into genetic or environmental, and is usually the first step in assessing the potential genetic basis for a human trait (48). Therefore the main finding of this study, that mortality rates of centenarian siblings in Okinawa are approximately half those of their general population birth cohort, is of particular interest and supports the existence of a substantial familial component to their longevity.

Clearly, families share common genes but families also tend to share common environmental habits such as eating habits, physical activity levels, socioeconomic factors, religious practices, and other nongenetic factors that impact survival (49,50). Therefore, it is plausible that shared environmental factors could have been at least partly responsible for the sibling longevity. However, the fact that this mortality advantage persists relatively undiminished for the entire life span is of substantial interest because most mortality differentials, particularly those due to environmental factors, diminish or disappear at older ages. These mortality differentials include mortality differences due to gender (51), ethnicity (52), dietary factors (e.g., cholesterol) (53), physical activity (54), income (55), and education (56). The relative homogeneity of the Okinawan population with respect to all of these environmental factors (43) lends credence to the hypothesis that familial factors of genetic origin are very important for human longevity.

Most members of this birth cohort were physically active farmers and had little education beyond primary school (57). This was also the case for centenarians in Okinawa, where 59.5% of men and 57.1% of women worked as farmers, with the next leading occupations being office workers for men (18%) and homemakers for women (11.4%). Most centenarians never had extensive education, with 18% of men and 42% of women never having had any formal education at all, and the majority never graduating from high school (58). Even today, income is the lowest of all Japanese prefectures (43).

The traditional Okinawan diet tended to be low-calorie, plant-based, and composed mainly of low-caloric-density foods such as sweet potatoes, other green and yellow vegetables, soy foods, fish, and limited amounts of boiled red meat (39–41,57,58). Consistent with their low caloric intake, older Okinawans share several characteristics of the caloric-restriction phenotype as part of their exceptional longevity phenotype, including short stature, low body weight, low body mass index, and high HDL (high-density lipoprotein) levels relative to other Japanese (39–41,58–64). A lower caloric intake compared with other age-matched Japanese appears to be a persistent characteristic of both past and current diets of older Okinawans, including centenarians, suggesting lifelong lower caloric intake in the Okinawans (39–41,58–64).

It is interesting, despite this relatively low caloric intake in older individuals, that the caloric restriction phenotype has been partially, but not completely, eliminated in post-Second World War birth cohorts after a transition to a diet higher in caloric density with a concomitant mild increase in caloric intake (39–41). This nutrition transition has been coupled to a relatively large decrease in physical activity, and the resultant positive energy balance is associated with higher body weight and body mass index in post-Second World War birth cohorts (39–41). However, the persistence of some of the characteristics of the caloric restriction phenotype, such as shorter stature and low risk for chronic age-related diseases, despite these environmental changes, suggests that genetic factors have played at least a partial role in the longevity phenotype in Okinawa.

The occurrence of the centenarian phenotype at the

highest prevalence in Japan, itself the world's longest lived country, is remarkable considering the socioeconomic disadvantages the Okinawans have had relative to other Japanese (43). The high prevalence of very old individuals suggests that other factors, such as low energy intake, healthy diets, physical activity, social customs, or genetics may have played a more important role in their survival advantage. However, the relative genetic and environmental contributions to the longevity phenotype are as yet unknown, and a weakness of the current study is that there are few specific data from the 1890 general population birth cohort with which to make comparisons. Moreover, although we have some socioeconomic data on centenarians themselves, we do not have specific data on the centenarian siblings to assess whether they had specific environmental advantages over the general population cohort.

Nevertheless, consistent with a significant genetic influence on longevity, Okinawans have varied little ethnically, historically having married within their own villages, and there is little evidence of substantial gene flow for centuries, resulting in what appears to be less genetic variability in Okinawans than in other Japanese (65). Therefore, with a higher inbreeding coefficient and a limited gene pool, the potential for clustering of genetic traits influencing the longevity phenotype in the Okinawans is pronounced.

The finding of a sustained mortality advantage over the entire life span has important implications for the study of human longevity. This sustained mortality advantage in centenarians and their siblings suggests that these survivors may have clustered genetic variants that facilitate a relative resistance to age-associated diseases and/or senescent processes. In support of this hypothesis, several candidate genetic variants associated with the exceptional longevity phenotype have been identified in nuclear genes in Okinawans (16,17) and relatively rare mitochondrial haplotypes appear to have clustered in Okinawans that might be linked to their exceptional longevity (65). Some of these nuclear genetic variants are implicated in inflammatory and/or autoimmune processes (16,17), which are candidate mechanisms for aging and age-related pathology (18), and some mitochondrial haplotypes have been linked to longevity in both Japanese and Finnish populations (66,80).

More evidence that the human longevity phenotype may have substantial genetic contributions comes from a recent demographic study of human mortality. Horiuchi and colleagues (68) suggest that most individuals who achieve average life span are susceptible to age-related chronic diseases that enhance midlife mortality risk, whereas those who survive to exceptional ages do so because of resistance to these diseases. This hypothesis is based on the observation that there are differential risk patterns for particular diseases at different ages. Causes of death at middle age are more likely due to chronic diseases such as coronary heart disease and cancer, whereas death at older ages is dominated by senescent processes and acute conditions, such as respiratory infections and accidents (e.g., falls).

Research on the morbidity profiles of centenarians by Evert and colleagues (69) also suggests that those who achieve centenarian ages have distinct phenotypes and possibly distinct longevity genotypes; 87% of male and 83% of female

centenarian individuals delayed (at least until age 80 years) or escaped clinical evidence of coronary heart disease, nonskin cancer, and stroke. The fact that some centenarians survive to their exceptional ages without clinically manifest chronic disease, and without autopsy evidence of cardiovascular disease or cancer, supports the existence of genetic factors that confer relative resistance to these diseases, and results in a higher probability of achieving exceptional old age (7,69). Indeed, evidence for the existence of SNPs associated with protection from cardiovascular disease that appears to confer exceptional longevity has recently been discovered in a population of Ashkenazi Jewish centenarians (70) and long-lived Japanese Americans (71).

The primary argument against a significant genetic contribution to exceptional longevity has been from the findings of twin studies that purport the heritable component to human life-span ranges from 20% to 30% (72,73). These findings, coupled with arguments from evolutionary biologists that there is little advantage for evolution to invest in genes that may confer survival beyond reproductive years, argue against the feasibility of conducting further investigations for genes that might confer relative resistance to age-related diseases and survival advantages. However, this argument rests on conclusions made from study populations that have not included a substantial portion of long-lived members (23). Small studies that lack a sufficient population of long-lived individuals will have insufficient power to detect longevity-related genetic effects. For example, the Swedish and Danish Twin Studies, from which much of this argument has been derived, had an average age of death that was little different from that of the average population, and lacked a large sample of long-lived individuals where longevity genes are more likely to cluster (72,73). Using the Swedish Twin Study and a more robust statistical model, Yashin and colleagues (20) concluded that half of the variation in susceptibility to mortality is genetic.

Moreover, studies that have included a significant number of long-lived individuals, and studies of sufficient size to have the statistical power to detect small- to medium-sized genetic effects, have found credible evidence for familial clustering of longevity. For example, a study of 78,994 individuals from the Utah Population Database (UPDB) who lived to at least age 65 years found significant evidence for the familial clustering of longevity (26). Among siblings of probands who had reached at least age 95 years, the relative risk of achieving a similar age in the UPDB was 2.30 (95% confidence interval, 2.08-2.56). There was also substantial evidence for Mendelian genetic modes of transmission of longevity.

A large study of the Icelandic population revealed that first-degree relatives of probands living to the 95th percentile had close to double the chance of achieving this age (45). A third study, which had methodology similar to ours, found that U.S. centenarian siblings had approximately double (women) to quadruple (men) the chance of living to age 90 years (21). These familial advantages are somewhat lower than the relative survival advantages found in the current study. Importantly, our study of Okinawan centenarian siblings was conducted in a genetically and socially homogeneous population, which may make it easier to disentangle genetic from environmental determinants of exceptional

human longevity. The high relative survival probabilities in the Okinawan centenarian siblings (which reflect the cumulative survival advantage over their lifetimes) may suggest a stronger genetic effect for longevity in the Okinawans. It is also possible that the genetic influences that could result in extended longevity in human populations might be easier to discern in the more socially homogeneous Okinawan population. Further research needs to be done to answer these questions. In any case, our study supports the aforementioned work (21,26,45) and lends credence to the hypothesis that familial factors that help to confer a survival advantage may be relatively widespread among human populations. Our study also supports the existence of longevity genes or genetic variants that cluster in very old individuals, and possibly cluster in some long-lived, genetically isolated populations.

These findings argue for further genetic research on exceptionally old individuals, such as centenarians, using linkage or association studies, particularly in genetic isolates. With the high relative survival probabilities seen in centenarian siblings, sufficient power for successful linkage analyses for longevity genes might be possible with a moderate number of sibling pairs, using anonymous polymorphic markers throughout the genome and/or markers for specific association studies of candidate gene loci (24). This approach has already mapped naturally occurring genetic variants (with human homologues) that contribute to longevity in fruit flies and mice (3).

The promise of family studies of longevity is supported by two recent studies using genome-wide scans for predisposing loci for exceptional longevity. Puca and colleagues (74) studied 308 individuals belonging to 137 long-lived sibships. Evidence for linkage was found for a chromosome 4 locus at D4S1564 with a nonparametric maximum logarithm of odds (LOD) or "MLS score" of 3.65 ($p = .044$). The MLS score is a statistical estimate of whether two genetic loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together. An LOD score of 3 or more is generally taken to indicate that two gene loci are close to each other on the chromosome and that the odds are a thousand to one in favor of genetic linkage. This nonparametric evidence was corroborated by a parametric analysis ($p = .052$). The segregation of exceptional longevity as an autosomal dominant trait in centenarian families argues for the existence of a gene or genes with a positive influence on longevity in this region of the genome.

Although genome-wide scans have been criticized due to numerous issues (including false-positive associations inherent in multiple comparisons) and to the fact that they can be subject to population stratification artifact, this largely depends on the type of genome-wide scan (e.g., family-based linkage vs case-control association study), methods used for statistical analysis, and the choice of study population (75). Family studies that use linkage designs overcome limitations of population stratification but are often underpowered due to difficulties in collecting adequate numbers of long-lived participants. Moreover, linkage-based studies, which look for co-inheritance of chromosomal regions with disease in families, tend to have less precise

localizing information, which can extend over millions of base pairs. Nevertheless, the Puca and colleagues (74) sibling linkage study was supported by another genome-wide linkage scan in 95 pairs of male fraternal twins concordant for healthy aging. The same region of chromosome 4 produced an LOD score of 1.67. It was the most promising region among six chromosomal regions identified, with LOD scores greater than 1.2 ($p < .01$) (76). Further genotyping of this area on chromosome 4 has yielded at least one candidate gene for human longevity, related to lower cardiovascular risk (77).

In contrast, genome-wide association studies using case-control design, in which long-lived individuals, such as centenarians, are matched with short- or average-lived controls, are an increasingly viable option to understand the allelic variation that underlies the longevity phenotype (75). Although complete genome sequencing is not yet feasible for large studies, it is now possible to partially scan the genome by genotyping large numbers of common SNP gene variants (gene variants that occur with at least 1% frequency) in genome-wide association studies (75). Whole-genome association studies provide more precise localizing information, often extending to only a few thousand base pairs, by detecting differences in frequency of genetic variants between unrelated individuals with a phenotype of interest and controls. These variants may themselves be of interest or they may be correlated with another nearby gene that contributes to the phenotype of interest. This is where long-lived, genetically homogeneous populations such as the Okinawans become particularly valuable, as opposed to more admixed populations, such as centenarian populations composed mainly of mixed immigrant populations (i.e., North America).

As mentioned, the Okinawans appear to be an isolated population, and this isolation has important implications for genetic studies (44,78). Large movements of people and subsequent gene admixture (mixing of two or more genetically distinct populations) are substantial confounding factors in genetic studies (44,75,78). Admixture can significantly influence relative allele frequencies, particularly when the immigrant and local populations are very different. Changes in allele frequency can be seen in just one generation that may result in artifactual difference in genetic studies (78). These changes are of particular concern in the United States because whites in the United States are a markedly admixed population from diverse European origins. Therefore, the genetic composition of a U.S. centenarian sample is likely to differ substantially from that of a younger control population simply due to different migration patterns over different generations (78). These problems do not exist in Okinawa, which has had extremely limited immigration. Inappropriate matching of cases and controls under even modest levels of population stratification can cause both false-positive and false-negative findings (78).

Conclusion

Genetic factors appear to be important lifelong modulators of human aging. Although many genes may be involved, there are likely at least three general types. One, long-lived humans may lack genetic polymorphisms that increase risk for premature mortality due to specific disease processes,

such as cancer or cardiovascular disease (67,79). Two, genes may exist that slow accumulation of damage to vital processes at molecular, biochemical, or physiological levels, which is supported by studies of mitochondrial genes (67), immune response/inflammation-linked genes (16–18), and insulin-signaling genes (15,81). Three, genes that result in more efficient repair of genetic, cellular, or tissue damage may enhance longevity (82,83).

The familiarity of exceptional longevity suggested by the current study supports further efforts to identify potential longevity genes in long-lived humans. This approach may be particularly helpful for investigating candidate gene polymorphisms discovered in lower organisms for potential applicability to human aging or age-related disease. Genome-wide scans using family-based linkage methods or case-control association methods are increasingly attractive approaches for uncovering the genetic basis of human longevity. Discovering genetic variants that modulate aging and have widespread effects on age-related diseases could potentially have an enormous impact upon human health.

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