Original Article

Secular trends towards delayed onsets of pathologies and prolonged longevities in Japanese patients with Werner syndrome

Makoto Goto[1,*], Masaaki Matsuura[2]

[1] *Division of Anti-ageing and Longevity Sciences, Department of Clinical Engineering, Faculty of Medical Engineering, Toin University of Yokohama, 1614 Kurogane-Cho, Aoba-ku, Yokohama, Japan;*
[2] *Department of Cancer Genomics, Cancer Institute, Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo, Japan.*

Summary **Recent cases of increasingly elderly Werner Syndrome (WS) patients have paralleled increased lifespan in the general population, however, historical temporal lifespan variations in WS have not yet been ascertained. To assess temporal changes in life-span and progeroid comorbidity in WS, all Japanese WS patients documented from 1966-2004 were analyzed for age at onset of diabetes mellitus (DM), malignancy, and death. Of 1,019 WS analyzed, average age significantly increased for all variables studied over the study period. Average age of onset of malignancy and DM in all WS increased from 35.8 to 48.8 years and from 34.9 to 39.7 years, respectively ($p < 0.001$), while age at death increased from 38.2 to 52.8 years ($p < 0.001$), *vs.* 71.7 to 82.7 years ($p < 0.001$) in the general population. Lifespan increases in WS and the general population suggest a common environmental influence. Unlike the general population, no gender-specific difference in life-span occurred in WS, suggesting a gender-specific differential environmental effect on mutated WRN. Identification of factors responsible for age differences could facilitate improvement in survival and ageing phenotypes of WS patients and the general population.**

Keywords: Diabetes mellitus, Environment, Genetics, Longevity, Werner syndrome

Introduction

Werner syndrome (WS: MIM#27770) is caused by autosomally-recessive inheritance of a mutated RecQ3 DNA/RNA helicase gene (WRN) and is characterized by a variety of clinical manifestations which mimic features of advanced ageing (*1*). Patients with WS usually develop normally early on, but experience premature termination of the teenage growth spurt. This is commonly followed by hierarchical deterioration of a variety of connective tissue systems resulting in physical symptoms such as gray hair, alopecia, skin atrophy, skin sclerosis, skin hyper/hypo-pigmentation, vocal cord atrophy, osteoporosis, sarcopenia, bilateral cataracts, metastatic subcutaneous calcification, and atherosclerosis. Other systems adversely affected include the endocrine system, resulting in type II diabetes mellitus (DM), hypogonadism, and thyroid disorders; the metabolic system, resulting in hyperlipidaemia, hyperuricaemia and hyaluronuria, and malignancy (particularly sarcomas); and to lesser degrees the immune system, resulting in excessive auto-antibody production, defective cytokine responses and natural killer cell activity; and the nervous system, resulting in cognitive disorders and brain atrophy (*2*,*3*). Mutation of the WRN helicase, therefore, primarily affects mitotic rather than post-mitotic cell systems. Death due to malignancy or atherosclerosis-related conditions such as myocardial infarction typically occurs in the late 40's (*4*,*5*).

Since the first description of WS by the German family physician Otto Werner in 1904 (*6*), other WS case reports have accumulated worldwide (*7*). Interestingly, the majority (~75%) of WS patients are of Japanese descent (*8*), likely due to the relatively high frequency of consanguineous marriage in rural areas and an extremely high prevalence (1:100) of heterozygosity in the general Japanese population (*9*). Approximately 10 WS patients per year are documented in Japan, while only 3.3 patients

**Correspondence to:* Dr. Makoto Goto, Division of Anti-ageing and Longevity Sciences, Department of Clinical Engineering, Faculty of Medical Engineering, Toin University of Yokohama, 1614 Kurogane-Cho, Aoba-ku, Yokohama 225-8502, Japan;
e-mail: goto@cc.toin.ac.jp

per year are reported outside the country.

Recently, numbers of more elderly WS patients (over 60 years of age) have been trending up in Japan in parallel with increased longevity in the general Japanese population, indicating a possible common environmental link. To determine the extent of influence of environmental factors on the genotypic and phenotypic expression of WS, we reviewed all WS case reports published in Japan since 1966 and assessed whether the pattern of clinical manifestations had changed over time.

Materials and Methods

The first case of WS in Japan was reported in 1917 (*10*), the first death in 1966 (*11*), and the first association of WS with malignancy in 1968 (*12*). We analyzed the clinical manifestations of WS as described in all papers published between 1966 and 2004. WS publications were selected through a citation index (Igaku-Chuo-Zasshi) and bibliographies of each report were extensively examined for additional references. For comparison of Japanese WS patients with those outside Japan, searches were performed through PubMed. Care was taken to thoroughly identify patient family details, personal histories, authors, institutions and demographic characteristics to avoid the inclusion of duplicate patient data.

As most patients were diagnosed clinically with WS, diagnoses given by the original authors were carefully re-evaluated based on the presence of the following phenotypes: unusual body habitus, bilateral cataracts, skin sclerosis, painful corns, sarcopenia, metastatic subcutaneous calcification, skin ulcers, DM, and hyperlipidaemia (*8,13*). Of all cases analyzed, 77 patients reported after 1985 were confirmed as having WRN mutations after 1996, the year in which WRN was first identified (*14*). We selected age of diagnosis for clinically overt stage DM, age of diagnosis of the first malignancy, and age at death, as relatively reliable manifestations or outcomes of WS. Although early onset of cataracts is the hall mark of WS, we excluded this criterion from our study because accurate determination of when onset occurred could not be achieved due to differences in reporting criteria between case studies. Body weight, height and body mass index (BMI) as calculated by height and weight, were evaluated to determine environmental effects on metabolic syndrome, including DM (*15*). We further examined if longer life-span was linked with slower progeroid outcomes and if earlier onset of clinical symptoms was associated with a shorter life-span. Since most symptoms characteristic of WS usually overlap with those of natural ageing at an early stage of life, most patients, family members and even doctors, do not acknowledge presence of the disease before the age of 36.7 ± 10.1 years (*2*) even if additional family members are affected. Reliable records, particularly of early pathophysiological manifestations of WS, are therefore limited.

Data source

A total of 1,019 Japanese WS patients reported in 468 publications between 1966 and 2004 (> 99% of all documented patients during this time frame) were analyzed to confirm age differences in the onset of DM, malignancy, and age at death. Age of DM onset was collected from 430 patients; age of onset of the first malignancy from 345 patients; and age at death from 219 patients. To examine possible trends in BMI changes, body weight and height measurements were also determined. Data from the general population was obtained through the Annual J Health and Welfare Statistics Japan (Annual J Health and Welfare Statistics Japan, 2007 and website *http://www.mhlw.go.jp-toukei-youran-aramashi-ichiran.pdf*).

Statistical analysis

Using multiple regression analysis, we investigated temporal effects on the age of onset of DM, malignancy, and death in WS patients between 1966 and 2004, adjusting for patient sex as a confounding factor. The regression models were based on the following equation:

$$Y = b0 + b1(Year - 1985) + b2(Sex)$$

where Y represents the published patient age, Year represents the calendar year of the initial publications for each WS patient, and Sex is represented by zero (0) for males and one (1) for females. Estimation of regression coefficients are represented by b0, b1 and b2, where b0 corresponds to the intercept and is interpreted as the mean male age in 1985, the mid point of the study period; b1 expresses the age change per year, and b2 represents the effect of gender by the difference in age between males and females. Analyses of variance (ANOVA) were performed to confirm the fitness of the regression model. Statistical analyses were performed with the statistical package STATISTICA.

Results

A total of 1,019 cases documented in 468 Japanese articles published between 1966 and 2004 were included in the analysis. Using multiple regression models, temporal effects on the age of onset of DM, malignancy and death in WS patients were determined. Table 1 indicates regression coefficients with standard error, *p*-values and variance analyses. The ANOVA indicated that all models fit the data well.

In 1985 which was the midpoint of the study, the average age of onset of DM in WS patients was estimated to be 38.8 years for males and 3.07 years younger for females ($p = 0.002$) (Figure 1A). The

Table 1. Regression analyses for sex adjusted period effects on age of onset for DM, malignancy and age at death in the patients with Werner syndrome

		Estimated coefficient	Standard error	*p*-value
Diabetes mellitus (*N* = 430)				
Intercept		38.81	1.513	< 0.000001
Year-1985		0.128	0.048	0.00894
Sex		-3.074	0.970	0.00165
	ANOVA	F(2,427) = 8.4021	*p* = 0.00026	
Malignancy (*N* = 345)				
Intercept		43.37	0.836	< 0.000001
Year-1985		0.352	0.069	0.000001
Sex		-2.548	1.125	0.0243
	ANOVA	F(2,342) = 15.513	*p* = 0.00001	
Death (*N* = 219)				
Intercept		46.30	0.949	< 0.000001
Year-1985		0.384	0.082	0.000005
Sex		-1.705	1.394	0.223
	ANOVA	F(2,216) = 11.389	*p* = 0.00002	
Death in general Japanese population				
Intercept		74.21	0.090	< 0.000001
Year-1985		0.288	0.0056	< 0.000001
Sex		5.881	0.127	< 0.000001
	ANOVA	F(2,75) = 2391.7	*p* < 0.000001	

Sex: male = 0, female = 1

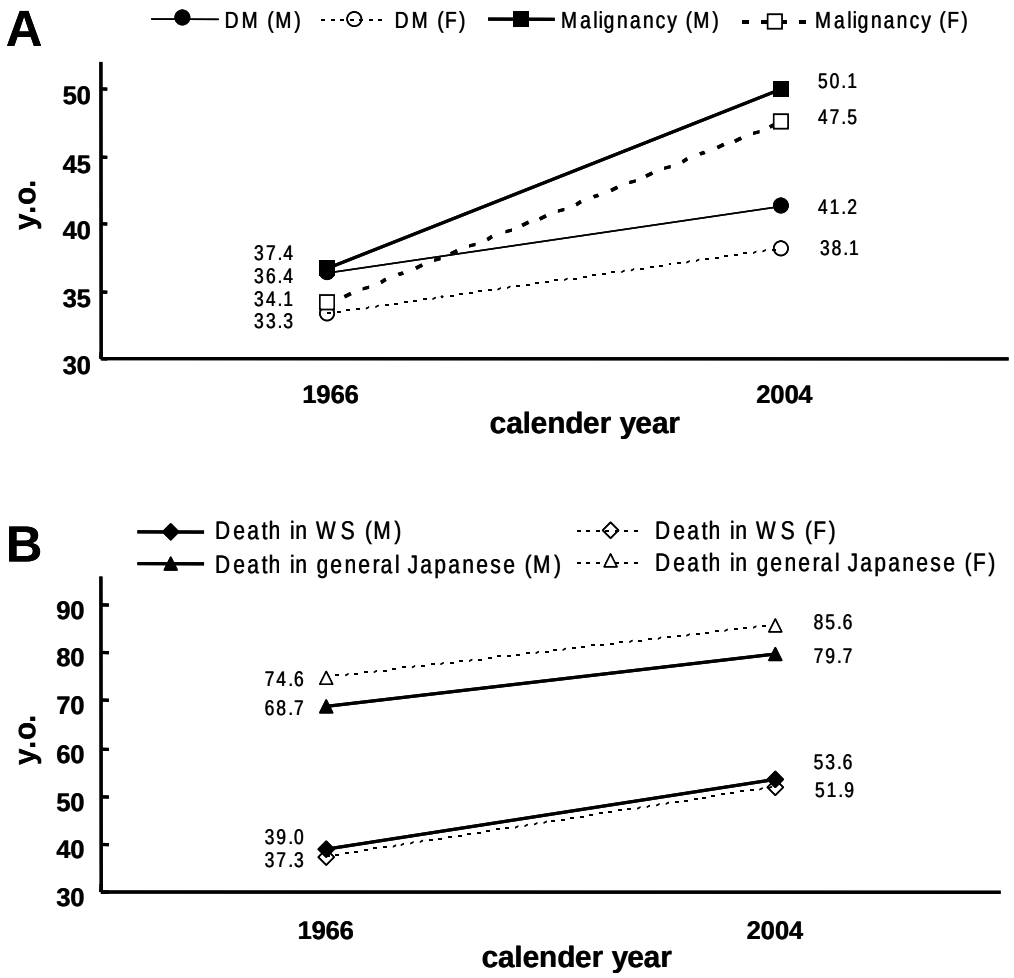

Figure 1. (A) Temporal trends of the age of onset of DM and malignancy, and (B) age at death in Japanese WS patients and the average life-span in the general population. Age of onset of DM and malignancy in male and female Japanese WS patients and age at death in WS patients and the general population are compared between 1966 and 2004.

incidence of DM in WS patients was ~70% for both sexes throughout the study period. A statistically significant increasing trend in the age of onset of DM in WS patients was observed in both males and females, with an estimated value of 0.128 years annually (p = 0.009). From the period of 1966 to 2004, therefore, the average age of DM onset in all WS patients increased by 4.8 years (from 34.9 years to 39.7 years).

Since BMI is an accurate indicator of DM in the general population, regression analyses of sex-adjusted temporal effects on body weight, height and BMI in WS patients were performed. Results in Table 2 show significant increases in body height ($p < 0.001$), weight ($p < 0.001$) and BMI ($p = 0.043$) in WS patients over time, in concert with the general Japanese population (Annual J Health and Welfare Statistics Japan, 2007 and website *http://www.mhlw.go.jp-toukei- youran-aramashi-ichiran.pdf*). Male WS patients, as with males in the general population, consistently surpassed female patients in body weight, height and BMI. However, BMI in WS patients in general is usually less than 18.1, and no significant difference in BMI was observed between patients with and without DM (data not shown). The estimated coefficients for DM were –0.61 (p = 0.405) for body weight, –0.20 (p = 0.782) for height, and –0.23 (p = 0.385) for BMI. The estimated coefficients of intercept, year and sex were almost identical between patients with and without DM.

With age of onset of the first malignancies, the regression curve patterns were similar to those of DM onset in regard to the annual age increase for both sexes throughout the analysis period, although the magnitude of the annual increase was larger (0.352 years annually, $p < 0.001$, Table 1) (Figure 1A). This amounts to a 13 year increase from the period of 1966 to 2004 (from 35.8 years to 48.8 years). Additionally, similar to DM, a gender difference in the age of onset of the first malignancies was noted, with a female onset age 2.548 years lower than that of the male average of 43.4 years at intercept (p = 0.024). The primary types of malignancies observed throughout the study period included a preponderance of rare tumors such as soft tissue sarcomas, malignant melanomas, thyroid carcinomas, meningiomas and hematologic disorders, as described (*4*).

A highly significant annual increase of 0.38 years in the age at death was seen in WS patients ($p < 0.001$), with average age at death of all patients increasing from 38.2 years to 52.8 years from 1966 to 2004. The increase was gender-independent, in sharp contrast to the general population, in which Japanese females live significantly longer than males (Annual J Health and Welfare Statistics Japan, 2007) (Figure 1B). From 1966 to 2004, the average life span of male WS patients increased from 39.0 years to 53.6 years, giving an average increase of the age at death of 14.6 years. In each year throughout the study period, the average life span of female WS patients was 1.705 years lower than that of males. For those with a molecular diagnosis of WRN, average age at death after 1985 was 50.7 years for males and 52.0 years for females, comparable to that reported by others (*16*). Fifty patients over the age of 50 with confirmed WRN mutations are still alive.

To compare results of WS patients with those of the general population, we examined age-adjusted temporal effects among the Japanese population during the same study period using National Statistics data (Annual J Health and Welfare Statistics Japan, 2007 and website *http://www.mhlw.go.jp-toukei-youran-aramashi-ichiran.pdf*). As seen in WS patients, a highly significant, though lower annual increase in the age at death in the general population was observed (0.288 years, $p < 0.001$, Table 1). From 1966 to 2004, average lifespan for both males and females in the general

Table 2. Regression analyses for sex adjusted period effects on body height, weight and BMI in the patients with Werner syndrome

		Estimated coefficient	Standard error	p-value
Height				
Intercept		152.01	0.479	< 0.000001
Year-1985		0.205	0.034	< 0.000001
Sex		-9.753	0.673	< 0.000001
	ANOVA	F(2,395) = 118.39	$p < 0.000001$	
Weight				
Intercept		40.97	0.483	< 0.000001
Year-1985		0.158	0.034	0.000044
Sex		-7.415	0.678	< 0.000001
	ANOVA	F(2,395) = 67.66	$p \leq 0.000001$	
BMI				
Intercept		17.63	0.176	< 0.000001
Year-1985		0.025	0.012	0.0426
Sex		-1.076	0.247	0.000017
	ANOVA	F(2,395) = 11.01	$p =$ 0.000022	

Sex: male = 0, female = 1

Table 3. Longevity of Werner syndrome patients

Reported year	Male			Female		
	Age at death	Cause of death	ID	Age at death	Cause of death	ID
1966-1985	70	AMI	WS61901			
1986-2004	79	malignancy	WS36101	69	still alive	unknown
	77	AMI	WS1801	63	AMI	WS9101
	69	AMI	WS1601	63	AMI	WS8101
	67	malignancy	WS1501	62	malignancy	WS15601
	67	malignancy	WS29901	61	malignancy	WS5701
	64	malignancy	WS1201	61	malignancy	WS1701
	63	AMI	unknown			
	63	malignancy	WS52301			
	63	malignancy	WS13201			
	62	malignancy	WS7801			
	62	malignancy	WS21701			
	62	malignancy	unkown			
	61	malignancy	WS23501			
	61	malignancy	WS23502			
	61	unkown	WS60901			
	61	unkown	WS60301			

AMI: acute myocardial infarction

population increased roughly ~11 years, from 71.7 years to 82.7 years.

Prior to 1985, no females and only one male WS patient aged over 60 were documented (Table 3) compared to 17 male and 6 female WS patients over 60 years after 1986. Strikingly, a 77-year-old male patient was diagnosed with WS by genetic analysis (mutation 4/4) (*2,17*) after 1986. Outside Japan, only one 62-year-old Irish male patient and one 63-year-old Dutch female patient were noted prior to 1985 (*18,19*), and only one 62-year-old Israeli female patient was reported after 1986 (*20*).

Two long-lived patients (WS1801, WS1501) were not diagnosed with WS prior to 35 years of age. In a few patients who had shorter life-spans of < 40 years, premature aging phenotypes typical of WS before age 10 were noted (data not shown). The percentage of WS patients suffering early death at < 40 years of age was 34.9% prior to 1985, and 13.3% after 1986 for both sexes. There was no significant difference between males and females in the frequency of early death.

Thus, although data is limited, there appears to be no correlation between delayed onset of WS-specific progeroid symptoms and a longer life-span or vice versa. The major causes of death in WS patients were malignancy (70%), atherosclerosis-related conditions such as myocardial infarction and cerebral infarction (20%), and infection (10%). The causes of death in WS patients were comparable in males and females prior to and after 1985 as described previously (*2*).

Discussion

There are several possible reasons why Japanese WS patients from both sexes similarly showed temporal-dependent delayed outcomes such as DM, malignancy and death. One explanation is that even in a genetically-defined disease such as WS, clinical phenotypes that mirror the natural ageing process in the general population may be strongly influenced by environmental/epigenetic factors in a similar fashion irrespective of sex. This is illustrated by the significant increase in longevity, body weight, height and BMI in WS patients from both sexes which was mirrored in the general population (Annual J Health and Welfare Statistics Japan, 2006 and website *http://www.mhlw.go.jp-toukei-youran-aramashi-ichiran.pdf*). With economic growth burgeoning in Japan from the mid 60's, developments in lifestyle, nutrition, hygiene and medical care improved population health as a whole, particularly in newborns and the elderly, resulting in a low percentage of neonatal death and increased longevity (Annual J Health and Welfare Statistics Japan, 2007). This rapid economic growth is a double-edged sword, however, as the prevalence of obesity, metabolic syndrome and malignancy among the general population also soared (*21*).

The annual increase in the age at death in WS patients (0.384 years) was statistically higher than that of the general population (0.288 years), based on a 95% confidence interval (Table 1). Environmental influences may therefore benefit WS patients more than the general population. This is supported by the observation that even though body weight, height and BMI in WS patients and in the general population increased significantly over time, BMI in WS patients was far below the normal range and may not be directly linked with the onset of DM as has been observed in the general population (Table 2). Recent environmental influences in Japan may therefore act in a beneficial manner for both WS patients and the general population prior to puberty, but may be detrimental, at least in relation to the onset of DM, after maturity.

Even though we do not have detailed data for the

age of onset of DM and malignancy in the general population, it is possible to estimate the trend over time based on population studies of the prevalence and incidence of DM (*22-25*) and malignancy (website *http://ganjoho.ncc.go.jp-public/statistics/backnumber/2000-en. html*). Population studies suggest that a recent trend in the general population of DM onset at younger ages is probably partly due to parallel increases in obesity among young people, which is in sharp contrast to WS patients.

The delay in progeroid outcomes in WS patients is likely not due to the simple technical advances in diagnosing DM and malignancy that have occurred over the last 40 years. If it had been, changes in the time of onset of both conditions would have been similar in both the general population as well as in WS patients, which was not the case. Additionally, most patients were diagnosed with DM before being diagnosed with WS (*2*). DM and malignancy are both hallmarks of WS and therefore monitoring for these conditions is more rigorous in WS patients than in the general population. It is likely, therefore, that detection of these conditions in WS patients would be enhanced, not delayed, as was seen.

Between 1966 and 2004, Japanese women were found to live ~7 years longer than men, a difference that was not maintained in WS patients. In 2004, male WS patients died at an average age of 53.6 years, compared to females at a similar age of 51.9 years. The lower female age at death may at least partly be due to the consistently lower age of onset of DM and malignancy seen in females compared to males. Thus, recent environmental factors may therefore favor male WS patients over female patients and the general population, which may suggest a gender-specific differential effect of the environment on mutated WRN. Another possibility is that female-specific genes (possibly hormone-related) are more negatively affected by the loss of WRN, although such evidence is lacking at present.

Although WS is an autosomal recessive disease that affects both sexes basically in a similar fashion, most clinical manifestations in WS are ageing-related phenotypes and as with general population ageing phenotypes especially longevity may favor female. Some phenotypic manifestations in WS could be regulated by both the loss of WRN condition which is environmentally dependent and also gender-related genes which are independent from the environmental effects. The gender-related genes may affect ageing on WS.

Finally, some of the ageing-associated phenotypes seen may relate directly to the loss of normal WRN function. Ageing is believed to induce genetic instability leading to cancer (*26*,*27*), therefore, the complete loss of WRN function may epigenetically and genetically impact other genes, promoters or proteins related to ageing-associated pathophysiology (*28*).

The present study suggests common as well as unique temporal environmental influences on hereditary disease and on the general population that affects ageing-associated phenotypic expression. The results also suggest that even in a genetically determined disease such as WS, the possibility of gene therapy-independent therapeutic intervention may exist. While this notion is highly speculative, the prospective cohort study by using the large number of mutation-proven Japanese patients may allow direct testing of these concepts in the future.

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