Review

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Werner syndrome: A changing pattern of clinical manifestations in Japan (1917~2008)

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Summary **As ~75% of the Werner syndrome (WS) patients recognized between 1904 and 2008 all over the world are of Japanese origin, the most case reports and clinical studies on WS has been published in Japanese journals. Thus, the detailed English-written clinical review on the recent WS case reports has been warranted. Although WS has been characterized by a variety of clinical manifestations mimicking premature aging, the recent longevity and delayed age-associated manifestations observed both from Japanese WS and general population may suggest a common environmental effect on some gene(s) other than WRN and may give us a newer pathophysiological look at WS and also natural aging through the molecular dysfunction of WRN.**

Keywords: Aging, cancer-prone syndrome, helicase, inflammation, longevity, premature aging, Werner syndrome

1. Introduction

Werner syndrome (WS:MIM#27770) is an autosomal-recessively inherited disease caused by the mutation of RecQ3 helicase (WRN) located at chr8p11-12 (*1-3*). WS patients are usually paid no special attention either from the family members or doctors concerning to any developmental abnormality until the usual premature termination of the teenage growth spurt and voice changes, followed by the age-related pathophysiology mimicking advanced aging (*4*). WS has been classified as an adult form of progeria, the representative natural model for human aging, a caricature of human aging, or as a segmental progeroid syndrome (*4-7*). The clinical manifestations recognized in WS, irrespective of ethnic origin, are commonly scheduled by hierarchical deterioration of the connective tissue system, the endocrine-metabolic system, and later to lesser degree the immune system, and the central nervous system (*4,5*). Like other helicases, the intact WRN helicase functions at the time of cell proliferation/division inside the mitotic cells, while the mutated/truncated WRN helicase protein does not (*8-10*). Thus, the systems/organs mainly consisting of post-mitotic cells like central nervous system and cardiac muscles may have at the most minor changes in WS. Actually no apparent association has been reported in WS with Alzheimer disease, Parkinson disease, and cardiomyopathy that may be frequently observed in elderly general population.

Since the first description of WS by Otto Werner in 1904 (*11*), additional WS case reports have accumulated worldwide, the majority being from Japan (*4,12*). So, we would like to review the details of the up-dated information written in Japanese to the non-Japanese readers (*13*).

2. Database

In the series of the previous reports, the literature has been searched for publications on WS through a citation index in and out of Japan between 1904 and 1995 (*3,4,14,15*). We have continuously searched the literatures and the referral cases between 1996 and 2008

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in the present communication. Bibliographies of each case were examined for additional references. Care was taken to exclude multiple reports of the same patient, recognized by details of family and personal histories and demographic characteristics.

Diagnosis of WS was based on the presence of 3 of 4 criteria under age 35 as described below (*3,4,14,15*): *i*) Characteristic habitus and stature:short stature and light body weight, slender extremities with stocky trunk and beak-shaped nose. *ii*) Premature senescence:bird-like appearance, alopecia/gray hair, skin hyperpigmentation, hoarseness, diffuse arteriosclerosis, juvenile bilateral cataracts and osteoporosis. *iii*) Scleroderma-like skin changes:atrophic skin and muscle, circumscribed hyperkeratosis, telangiectasia, tight skin over bones of feet, skin ulcers and localized calcification. *iv*) Endocrine-metabolic abnormalities: diabetes mellitus (DM) and hypogonadism. Diagnosis of neoplasia was as given by the original authors.

Approximately 200 Japanese WS patients (WS0101-WS60001) diagnosed by our criteria were further confirmed by the loss of intact WRN protein and the presence of WRN mutations as previously reported (*3,16,17*). B-lymphoblastoid cells and skin fibroblasts from ~200 WS patients and their family members are deposited at RIKEN Bio-Resource Center (Tsukuba Japan) as Goto Collection of Werner Syndrome and can be used by researchers upon request (*http://biolod.org/class/cria304u12i/Goto_Collection_EBV_transformed_B_cell_lines_ and_primary_fibroblast_derived_from_Werner_syndrome_patient*).

3. Brief history of clinical characterization of Werner syndrome

The history of WS research begins with the publication of a doctoral dissertation by a German general physician (later an ophthalmologist) at the University of Kiel in 1904, Otto Werner (*11*). He described several progeric manifestations, in addition to skin sclerosis and bilateral juvenile cataracts. Werner carefully differentiated the clinical manifestations in his patients from those with similar phenotypic manifestations formerly described by Rothmund (*18*), later known as Rothmund-Thomson syndrome (RTS: MIM#26840; *18-20*). Interestingly, we found the mutation of RecQ4 (RECQL4) located at chr 8p24.3, belonging to the same RecQ helicase family as WS causes RTS (*21*).

In Japan, Ishida, an ophthalmologist in Kyoto University, reported the first probable Japanese case of WS in 1917 (*22*), followed by a continuous publication of Japanese cases up to 1,128, while a total of 359 outside of Japan at the end of 2008 (Figure 1) (*4,12,23*). The number of patients associated with malignancy (between 1907 and 2008) was shown in the parentheses within the table in Figure 1.

Thirty years later from Werner's original observation, the door to WS research was reopened by two New York internists, Oppenheimer and Kugel, in 1934 and 1941 (*24,25*). They coined the name: "Werner's syndrome" and published the first necropsy case. The first reference of the rare malignancy in WS, fibroliposarcoma, was reported by Agatson and Gartner in 1939 (*23,26*). A Boston internist, Thannhauser reviewed WS and RTS as the discrete syndromes in 1945 (*20*), and Seattle-based geneticist group, Epstein *et al*. released a landmark overview on 125 cases of WS including 8 Japanese cases in 1966 (*5*).

Since the Ishida's first case report, the first necropsy Japanese WS case was documented in 1966 by Hamada (*27*), followed by the first association case report of WS with malignancy: malignant melanoma in 1968 by

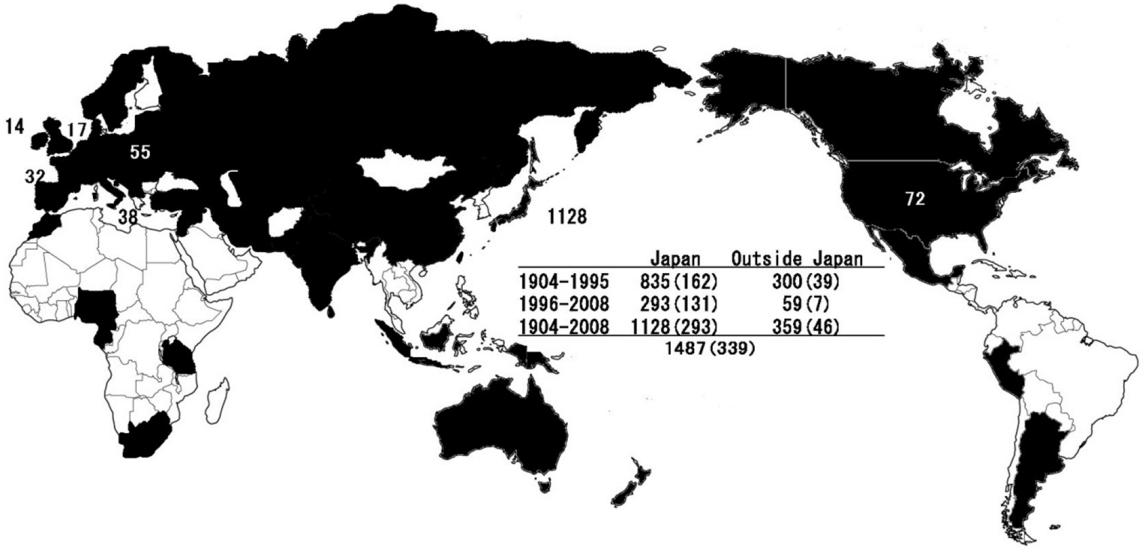

Figure 1. World distribution of Werner syndrome. The country with at least one reported patient is shaded. The number indicated for each country is the number of reported patients. The table within the figure indicates the number of reported patient for the respective term in and outside Japan. The number of malignancy associated with the patients is shown in the parentheses.

Koga (*23,28*). We proposed in 1981 for the first time the initial screening criteria for the possible WS patient as described (*3,4,14,15*).

4. Changing clinical manifestations

4.1. *Characteristic bird-like appearance and body habitus* (100% at age 35 years)

Short stature, light body weight, and stocky trunk with extremely thin extremities were noted in all the patients at age 26 years old. These characteristic appearance including bird-like faces, extremities mimicking Cushing syndrome or Klinefelter syndrome is still a hallmark of WS (*4,12,14*). Body size (height: 122-161 cm and weight: 19-52 kg) is also still small, but has been expanding in concert with the growing constitution in general Japanese population. Some patients exceeded 177 cm high and weighed over 70 kg.

4.2. *Premature senescence* (100% at age 35 years)

Gray hair/alopecia, bilateral cataracts, hoarseness, osteoporosis with osteosclerosis, and atherosclerosis are the hallmarks of WS. Interestingly, osteoporosis is usually more pronounced in postmenopausal women than men in general population, but this is not the case in WS. WS men have severer osteoporosis, either peripheral or vertebral, than women (*29,30*). Osteoarthritis, one of the commonest features in a general elderly population, has not been frequently reported in WS (*31*).

4.3. *Scleroderma-like skin changes* (100% at age 35 years)

Skin atrophy, skin sclerosis, skin ulcers, hypo/hyper-pigmentation, telangiectasia, sarcopenia, subcutaneous calcification, painful corns, and flat feet were included. Skin sclerosis such as extremely tight skin over malleoli and painful corns: the historical hallmark of WS has been rarer recently, though the reason is unknown. The skin ulcers in WS especially of lower extremities were induced by the daily mechanical stress in combination with the decreased cellular replicative potential and the subcutaneous fat defect (*14,32,35,36*). The skin ulcers in WS have been still well-known as the most incurable and occasionally leading to the amputation of the legs resulted from gangrene among dermatologists and orthopedic surgeons. Neither skin ulcers nor subcutaneous calcifications is usually associated with natural aging. Approximately 25% WS patients escape from skin ulcers (*32,35*). As described above, subcutaneous calcification, especially along the Achilles tendon is the must for diagnosing WS (*36*). So-far, all the mutation-proven WS have the Achilles tendon calcification, although relatively mild in some cases

4.4. *Endocrine-metabolic disorders* (type II DM, primary/secondary hypogonadism, thyroid hyper/hypo-dysfunction, hyperuricemia and hyper- lipidemia; 80% at age 35 years old)

The frequency of DM among Japanese WS patients has been constant in contrast to the rapid increase among general Japanese population. Of particular interest, the age at onset of DM in WS has delayed (*12*). The average age of onset of DM in WS was 33.7 years old in 1966, while 39.7 years old in 2004 and 39.3 years old in 2008. Since body mass index (BMI) was an accurate indicator of DM in the general population, the BMI in most WS patients was constantly below 22 (*12,33,37*), though all the WS patients showed an intravisceral fat accumulation revealed by MRI examination (*33,38*).

The serum adiponectin level decreased and the TNFα level increased in diabetic patients in general. In WS, adiponectin was significantly suppressed and TNFα is significantly enhanced compared with the controls. In addition, treatment of DM by pioglitazone normalized adipocytokines in WS (*38*). Recently, the WS patients associated with NASH (non-alcoholic steatohepatitis) have been reported (*39*).

Hypogonadism from both sexes was observed in 40%. Of special interest, one of the most prominent features in normal elderly male: prostate hypertrophy has never been recognized in WS. The thyroid dysfunction either of hyper-, hypo-function or malignancy that had been observed in the ~15% among WS patients has been rarer (*15*). All types of hyperuricemia were found in 10% in WS, though hyperuricemia was not usually associated with normal aging (*40*). Some patients had a history of gouty attack (*41*). Hyperlipidemia, characterized by hypertriglyceridemia was still a biochemical hallmark of WS (*4,37*).

The pathogenetical concept of atherosclerosis has been changing, though hyperlipidemia is still a risk factor for atherosclerosis. Atherosclerosis has been viewed as a sort of 'silent auto-inflammation' caused by the pro-inflammatory cytokines produced by macrophages that phagocytose modified lipoprotein particles (*42*). Interestingly, one autopsy case of 51 years old female WS patient who neither had dyslipidemia nor DM showed mild atherosclerosis similar to her age (*43*). She died of myelodysplastic syndrome (MDS).

Since the first description by Tokunaga *et al.*, the elevation of hyaluronan both in the urine and the serum has been constantly reported in WS. WS has been formerly inadvertently classified as a new group of hereditary mucopolysaccharidosis (*44-46*). Hyaluronan elevation either from urine and serum has been believed as a biomarker of normal aging and progeroid syndromes such as WS and progeria (*46-49*) and the International Registry of Werner syndrome included hyaluronuria for diagnosing WS (*http://www.pathology. washington.edu/ research/werner/registry/diagnostic.html*). However, the excessive production

of hyaluronan has been widely reported in other inflammatory conditions such as rheumatoid arthritis and liver diseases (*50-52*). The increased serum/urine hyaluronan level in aging and WS has been recognized as the result from chronic inflammation (*48,49,53,54*).

4.5. *Immune system disorders*

Immune system has been believed as the most sensitive organ to normal aging (*55-60*). At the age of 35 years, Approximately 80% of the WS patients show signs of mild immune abnormalities. A deficiency in so-far unidentified T cell subsets similar to the healthy nanogerian was detected in WS (*56*). Decreased natural killer cell activity, which recovered after the interferon stimulation, was observed in most WS patients (*57*). Most patients had low titers of several autoantibodies, including IgG anti-double-stranded DNA antibody, anti-nuclear antibody, and rheumatoid factor, as is usually observed in the healthy population over the age of 60 years (*58,59*). The autoantibody specific to autoimmune systemic sclerosis: anti-topoisomerase I (Scl-70), has never been detected in WS, although antinucleolar antibody of an undefined type was detected in some cases (*59,60*). Interestingly, a small percentage of the patients had autoimmune diseases, including Graves' disease, systemic lupus erythematosus, Sjogren's syndrome, and autoimmune hepatitis (*60*). However, WS patients were not abnormally sensitive to bacterial or viral infection at any stage of their life, even though the third major cause of death in WS is bacterial pneumonitis similar to the general Japanese population (*4*). The various types of low titer of auto-antibody production have been presumed as the result from the imbalance between inflammatory/anti-inflammatory responses against natural/modified antigens.

We have proposed the sub-normal immune system in WS may induce 'silent inflammation', 'inflammaging', or para-inflammation normally responsive to the daily infectious attacks and persistent oxidative stress, leading to the pathophysiology of DM, atherosclerosis, malignancy, auto-antibody production, and hyaluronan production (*48,50,52-54,61*).

4.6. *Nervous system disorders* (frequency: 40% at age 35)

WS has been believed to have a relatively normal central nervous system, consisting of mainly post-mitotic cells that may escape from WRN helicase dysfunction (*62*). However, with the recent advance of medical devices including computed tomography and magnetic resonance imaging (MRI), brain atrophy has been observed in 40% of WS patients, even before the age of 40 years (*4,63,64*). At least 3 WS patients had senile dementia due to subcortical arteriosclerotic encephalopathy or multiple meningioma, but not of the Alzheimer type by clinical determination and autopsy (*65-67*). Of great interest, 10% patients had schizophrenia of paranoia type at the age of ~37 years (*4*). Either bipolar or monopolar mood disorder has been rare in WS (*68*). Although ~10% WS had hearing loss as a result of otitis media infection and ~15% mental retardation before 1970, both symptoms have never been reported recently.

4.7. *Changing pattern of malignancy*

The average age of onset of malignancy in WS was 36.9 years old in 1966, while 48.8 years old in 2004 and 48.9 years old in 2008. As already reported, non-epithelial neoplasms including soft-tissue sarcoma (STS), osteosarcoma, malignant melanoma, benign meningioma, and myeloid disorders are still a hallmark of WS as listed in Table 1 (*23*). WS has been classified as a member of hereditary cancer-prone syndrome. The ratio of epithelial to non-epithelial cancers was about 1:1.5 instead of the usual 10:1. Thyroid carcinoma and malignant melanoma have been frequently associated with Japanese WS as was reported (*69*). Among epithelial neoplasms three WS were associated with pulmonary carcinoma that has never been reported before (*23*), though neither additional prostatic nor colorectal carcinoma has been reported as shown in Table 1.

Multiple primary neoplasms or myeloid disorders are also a hallmark in WS (Table 2) (*23*). Twenty five Japanese with WS had primary neoplasms, or neoplasia with MDS. Thyroid carcinoma, malignant melanoma, meningioma, MDS, and MFH (malignant fibrous histiocytoma) were the frequent counterpart in the multiple primary neoplasms. Of note, six primary neoplasms were found in a Japanese man with possible

Table 1. Neoplasms in Japanese Werner syndrome (1996~2008)

Diagnosis	No.
Non-epithelial	
Soft-tissue sarcoma	
MFH*	8
Others	12
Osteosarcoma	6
Malignant melanoma	18
Meningioma	9
Hematologic disorders	
AML**	4
MDS***	11
Others	8
Epithelial	
Thyroid	9
Liver	6
Skin	5
Lung	5
Others	30
Grand total	131

*MFH: malignant fibrous histiocytoma. **AML: acute myelogenous leukemia. ***MDS:myelodysplastic syndrome.

Table 2. Multiple primary neoplasms or myeloid disorders in Werner syndrome

Case	Mutation*	Sex	Diagnosis (age)
WS15001	6//6	M	Malignant melanoma, conjunctiva + osteosarcoma (all at 52)
WS32201	?//?	M	Malignant melanoma, acral lentigious (21) + malignant melanoma,intranasal (31)
WS31801	?//?	M	Malignant melanoma + multiple myeloma (all at 56)
WS25101	?//?	F	Malignant melanoma + leiomyosarcoma (all at 55)
WS57501	1//?	F	Pancreas carcinoma (60) + malignant melanoma (61)
WS32701	?//?	F	Thyroid, papillary + Bowen disease (all at 54)
WS33201	?//?	F	Thyroid, medullary (38) + fibrosarcoma (43)
WS10201	6//6	F	Thyroid, follicular (43) + ureter carcinoma (50) + adrenal carcinoma (50)
WS65001	?//?	M	Thyroid, type unknown(46) + meningioma (49)
WS10501	6//6	F	Thyroid, type unknown + hepatocellular carcinoma + MDS** (all at 56)
WS23801	?//?	M	Thyroid, type unknown + meningioma + MDS** (all at 62)
WS24701	?//?	M	MDS** (53) + meningioma (54)
WS35801	?//?	F	Meningioma + osteosarcoma (all at 58)
WS24101	?//?	F	Meningioma (63) + hepatocellular carcinoma (66) + cholangiocarcinoma (66)
WS32301	?//?	M	Fibrosarcoma + MFH*** (all at 51)
WS26401	?//?	M	MFH*** + malignant peripheral nerve sheath tumor + osteosarcoma (M, all at 58)
WS0402	4//4	F	SCC****, skin (53) + MFH*** (55) + bladder carcinoma (56)
WS24001	4//4	F	Breast (40) + MDS** (55)
WS36001	?//?	F	Malignant peripheral nerve sheath tumor + olfactory neuroblastoma (all at 41)
WS32601	?//?	M	Pheochromocytoma + malignant adrenal gland tumor (all at 55)
WS36101	?//?	M	"SCC****, oral soft palate (69) + left edge tongue (74) + right edge tongue (75) + hard plate (79) + esophagus (79) + ureter, tansitional cell carcinoma (82)"
WS56201	?//?	M	Bowen disease + SCC**** (all at 70)
WS52801	6//6	F	Uterine carcinoma (40) + leiomyosarcoma (52)
WS17601	?//?	M	Hepatocellular + basal cell carcinoma (all at 44)
WS14701	4//4	M	Gastric + renal carcinoma (all at 45)

*See details in Table 3; **MDS:myelodysplatic syndrome; ***MFH:malignant fibrous histiocytoma; ****SCC:squamous cell carcinoma.

WS, though the WRN mutation in the patient was not examined (*70*).

5. Genetics

5.1. *Geographical distribution and mutation type*

WS is a genetic disease transmitted by autosomally recessive inheritance (*14*). Although consanguineous marriage especially in rural areas may still contribute the relatively high incidence of WS in Japan, consanguinity (mostly first-cousin marriage) was noted in only ~45% among ~200 mutation-proven WS patients. In addition, familial occurrence has been infrequent since 1996. Most cases recently reported are sporadic. The patients have been reported from all area of Japan and outside of Japan (Figure 1), while there are still several clustering areas in Japan as have been already reported (*4,14,71*).

Since the discovery of WRN gene in 1996 (*2*), the mutation type of the responsible gene:WRN has been reported in and outside of Japan. According to the International Registry of Werner syndrome (*http://www.pathology.washington.edu/research/werner/registry/diagnostic. html*), the number of the mutation type has accumulated up to ~100 worldwide. Approximately 200 Japanese WS patients (WS0101-WS61901) diagnosed by our criteria were further confirmed by the loss of intact WRN protein and the presence of WRN mutations (*3,16,17*). Although the precise mutation in ~15% patients with defective WRN protein is not identified yet, the mutation types so-far recognized in Japan are quite different from those outside Japan as shown in Table 3. Interestingly, the majority of the Japanese patients with WS have mutation type 4, that has never been found in the non-Japanese WS.

Japan is the largest producer of WS (*4,14,71*), probably because of an extremely high incidence (1:100) of heterozygous carriers in Japan (*14,71,72*). Although few patients with WS have been reported in ethnically similar Asian countries such as Korea, the incidence of heterozygous carriers in general population in the representative 3 areas of Korea (Seoul, Pusan, and Gwangju) was less than 1:1,000 (*73*, personal communication from Drs. F. Takeuchi and A. Park).

5.2. *Changing pattern of longevity*

Like the decreased life-span of the cultivated and *in vivo* skin fibroblast, the WS patients have been believed to have a shorter life-span than normal (*34,74,75*). This notion may be generally true. However, the increase in

Table 3. Mutations in Werner syndrome in Japan

Nomination	Site (nucleotide no.)	Codon	Mutation type	Nucleotide sequence	Comments	Predicted protein (a.a)	% Mutated alleles in WS
Mut1	4,144	1,305	substitution	CGA --> TGA	nonsense	1,304	10
Mut4	1-bp upstream from 5' end of exon 26	1,047-1,048	substitution	aatagGGTAGA --> aatacggtaga	exon skip and frame shift	1,060	52.3
Mut5	4,146	1,305	1-bp deletion	CGAGCA --> CGAAGC	frame shift	1,017	1.4
Mut6	1,336	369	substitution	CGA --> TGA	nonsense	368	18.9
Mut7	3,677	1,149	1-bp deletion	GAGCGA --> GGCAGG	frame shift	1,160	0.9
Mut8	7-bp upstream from 5' end of exon 30(3690-3691)	1,153-1,154	substitution	tttgttcagATT --> ttagTTCAGATT	frame shift	1,162	0.6
					nonsense	462	0.6
Mut9	1,620	463	substitution	TAT --> TAA			
Mut10	733-734	168	2-bp deletion	AAGCTG --> GCTGAA	frame shift	176	0.6

*See details in Table 3; **MDS:myelodysplatic syndrome; ***MFH:malignant fibrous histiocytoma; ****SCC:squamous cell carcinoma.

Table 4. Association of the earliest progeroid symptoms with life-span

Onset (y.o)	Progeroid symptoms	Death (y.o)	Cause of death	ID/ Sex	Mutation*
4	Cataract	23	Cerebral bleeding	WS15701F	6//?***
7	Cataract	> 61	Still alive	WS0402F	4//4
8	Hoarseness	> 61	Still alive	WS8401M	4//4
8	Characteristic habitus	53	AMI**	WS5001F	4//4
10	Scleroderma	46	Malignancy	WS14701M	4//4
10	Characteristic habitus	51	Malignancy	WS12501M	1//4
10	Characteristic habitus	57	Malignancy	WS10001M	1//1
10	Characteristic habitus	77	AMI	WS1801M	4//4
11	Skin atrophy	39	Infection	WS53101F	1//1
11	Hyperkeratosis	53	AMI	WS2101F	4//4
12	Cataract	44	AMI	WS7001M	4//4
13	Cataract	49	Infection	WS0101M	1//4
13	Hoarseness	57	Malignancy	WS0401M	4//4
13	Characteristic habitus	70	AMI	WS61901M	4//4
14	Characteristic habitus	45	Malignancy	WS4401F	4//4
15	Cataract	39	Malignancy	WS23703M	4//6
15	Characteristic habitus	58	AMI	WS4801M	4//6
15	Characteristic habitus	59	Infection	WS0801F	4//4
15	Skin ulcer	61	Malignancy	WS1701F	6//6
16	Short stature	38	Infection	WS8601F	1//1
16	Skin ulcer	46	AMI	WS15301M	4//4
17	Cataract	59	AMI	WS6901F	6//6
20	Cataract	49	Infection	WS11201M	10//10
20	Cataract	55	Malignancy	WS11502F	6//6
20	Characteristic habitus	57	Malignancy	WS16001F	4//?
21	Cataract	47	Malignancy	WS9301M	4//4
22	Cataract	43	AMI	WS10402M	4//4
24	Diabetes mellitus	55	Malignancy	WS24002F	4//4
24	Cataract	43	Malignancy	WS24001M	4//4
25	Cataract	45	Malignancy	WS20001F	4//4
27	Gray hair	42	Malignancy	WS6201F	7//7
27	Skin ulcer	52	Malignancy	WS15001M	6//6
28	Cataract	56	Malignancy	WS55201M	4//4
29	Cataract	61	Malignancy	WS57501F	1//?
30	Cataract	38	Malignancy	WS5901M	6//6
30	Cataract	48	Malignancy	WS25402M	5//5
30	Cataract	50	Malignancy	WS10201F	6//6
30	Cataract	66	AMI	WS51002M	4//4
32	Cataract	50	Malignancy	WS25401F	5//5
32	Cataract	56	Malignancy	WS10501F	6//6
35	Cataract	43	Malignancy	WS50902F	1//6
35	Cataract	49	Infection	WS51001F	4//4
36	Cataract	55	Malignancy	WS16301F	6//6
37	Malignancy	37	Malignancy	WS8602F	1//1
37	Cataract	> 65	Still alive	WS4702F	6//6
37	Cataract	70	AMI	WS4701F	6//6
38	Gray hair	52	Malignancy	WS9501M	6//6
39	Cataract	52	AMI	WS4704M	6//6
40	Characteristic habitus	> 62	Still alive	WS6701F	4//4
41	Malignancy	46	Malignancy	WS15501F	4//4
44	AMI	63	AMI	WS9101F	4//4
52	Cataract	59	AMI	WS8801F	1//4
53	Skin ulcer	63	Infection	WS12401F	4//4
63	Malignancy	63	Malignancy	WS52301M	4//4

*See details for Table 3; **AMI: acute myocardial infarction; ***//?: mutation unidentified.

Table 5. Longevity of heterozygote members in Werner syndrome family

WS/ Sex	Age at death	Cause of death	ID	Mutation*
WS0101M	97	Malignancy	Grand father	4//w**
WS7901F	92	Infection	Mother	?***//w
WS4401M	92	Malignancy	Father	4//w
WS0101M	>90	Still alive	Father	1//w
WS0801F	90	Infection	Grand mother	4//w
WS0801F	90	Malignancy	Mother	4//w
WS61901M	90	Infection	Mother	4//w
WS5701F	90	Cerebral bleeding	Mother	4//w
WS4501M	90	Infection	Grand father	?//w

*See details for Table 3; **w: wild type; ***?: mutation unidentified.

the number of elderly WS patients (age > 63 years) in Japan paralleled the increased longevity in the general population, as described in Table 4. Death occurred on an average of 52.8 years in 2004 (82.7 years in general population) and 55.0 years in 2008 (82.7 years in general population), although the life-span of WS was ~38.2 years in 1966 (71.7 years in general population) (*12*). The major causes of death are still malignancy and myocardial infarction (*4,5,12,14,15*). Interestingly, the way of the appearance of age-related pathophysiology followed by death observed in WS was similar irrespective of the ethnic origin and mutation type (*4,5,15*).

5.3. *Does early pathophysiology determine the life-span?*

Analyses of the way how aging-related conditions may rapidly arise in WS may give us the fundamental insight into the pathophysiological mechanisms of human aging. Since the most clinical manifestations characteristic in WS usually overlap with those of natural aging process at an early stage of life, most patients, family members and even doctors do not acknowledge the presence of the disease before the age of 36.7 ± 10.1 years (*4*), even if additional family members are already recognized as affected. The parents of children with WS may in some time recognize the abnormality either by their lack of the prepubertal growth spurt, the loss of sex maturation and voice change or the early onset of cataract. Reliable records, particularly of early pathophysiological manifestations of WS, are therefore limited. As listed in Table 4, we examined in mutation-proven WS; *i*) if longer life-span (age > 63years) was linked with slower progeroid outcomes (age < 40years), and *ii*) if earlier onset of clinical symptoms (age < 13years) was associated with a shorter life-span (age < 40years). These results may suggest a possible common environmental/epigenetical link between the longevity in WS and the general population (*12*).

Several long-lived patients (age > 63 years old) were not diagnosed with WS prior to 35 years of age. In a few patients who had shorter life-spans of age < 40 years, premature aged phenotypes typical of WS before age 10 were noted. The percentage of WS patients suffering from 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. The major causes of death in WS have been malignancy, acute myocardial infarction (AMI) and infection; similar to the general population.

Thus, although data is limited, there appears to be no clear-cut correlation between delayed onset of WS-specific progeroid symptoms and a longer life-span or *vice versa*.

5.4. *Family analysis: Does WRN heterozygote contribute longevity?*

The longevity (> 90 years old) members have been sometimes encountered among heterozygous carriers in WS families as listed in Table 5.

Although the data is still limited and we do not know if WRN heterozygosity may contribute longevity, WRN has been recently reported to modulate mitochondrial ROS (reactive oxygen species) production by the repression of HIF-1 (hypoxia inducible factor-1) activity (*76*). So, the 50% WRN function may moderately suppress ROS production leading to longevity. Obviously, this notion is highly speculative and further study may be required.

6. Conclusion

We should bear in mind that as the rapid improvement and changes in the average life-span and life-style in general population all over the world, the life-span and clinical manifestations even in the genetically-determined disease like WS may change extensively as already described (*12*). This may suggest the possible interventional treatment for the clinical changes in WS, age-related diseases in the general population and even pathophysiology of natural aging.

Although the recent delayed onset of typical progeroid phenotypes in WS caused by the loss of function of WRN may be explained by the environmental changes including life-style and medical improvement, genes normally cooperated with WRN may possibly

contribute unexpected phenomenon . While this notion is highly speculative, the *in vitro* molecular studies and the prospective cohort study by using the larger number of mutation-proven Japanese patients may allow direct testing of these concepts in the future.

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References

1. Goto M, Rubenstein M, Weber J, Woods K, Drayna D. Genetic linkage of Werner's syndrome to five markers on chromosome 8. Nature. 1992; 355:735-738.
2. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD. Positional cloning of the Werner syndrome gene. Science. 1996; 272:258-262.
3. Goto M, Imamura O, Kuromitsu J, Matsumoto T, Yamabe Y, Tokutake Y, Suzuki N, Mason B, Drayna D, Sugawara M, Sugimoto M, Furuichi Y. Analysis of helicase gene mutations in Japanese Werner's syndrome patients. Hum Genet. 1997; 99: 191-193.
4. Goto M. Hierarchical deterioration of body systems in Werner's syndrome: Implications for normal ageing. Mech Age Dev. 1997; 98:239-254.
5. Epstein CJ, Martin GM, Schultz AG, Motulsky AG. Werner's syndrome. A review of its symptomatology, natural history, pathologic features, genetics and relationship to natural aging process. Medicine (Baltimore). 1966; 45:177-221.
6. Martin GM. Genetic syndrome in man with potential relevance to the pathobiology of aging. Birth Defects Orig Article Ser. 1978; 14:5-39.
7. Martin GM. Genetics and aging: the Werner syndrome as a segmental progeroid syndrome. Adv Exp Med Biol. 1985; 190:161-170.
8. van Brabant AJ, Stan R, Ellis NA. DNA helicases,genomic instability, and human genetic diseases. Annu Rev Genomics Hum Genet. 2000; 1:409-459.
9. Stano NM, Jeong Y-J, Donmez I, Tummalapalli P, Levin MK, Patel SS. DNA synthesis provides the driving force to accelerate DNA unwinding by helicase. Nature. 2005; 435:370-373.
10. Matsumoto T, Shimamoto A, Goto M, Furuichi Y. Impaired nuclear localization of defective DNA helicases in Werner's syndrome. Nat Genet. 1997; 16:335-336.
11. Werner O. On cataract in conjunction with scleroderma. Doctoral dissertation. Kiel University. Schmidt and Klaunig, Kiel, Germany, 1904.
12. Goto M, Matsuura M. Secular trends towards delayed onsets of pathologies and prolonged longevities in Japanese patients with Werner syndrome. BioSci Trends. 2008; 2:81-87.
13. Faragher R, Goto M. An international effort to cure premature ageing. BioSci Trends. 2007; 1:66-67.
14. Goto M, Tanimoto K, Horiuchi Y, Sasazuki T. Family analysis of Werner's syndrome: A survey of 42 Japanese families with a review of the literature. Clin Genet. 1981; 19:8-15.
15. Goto M. Clinical characteristics of Werner syndrome and other premature aging syndromes: Pattern of aging in progeroid syndromes. In: From premature gray hair to helicase-Werner syndrome:Implications for aging and cancer. Gann Monograph on Cancer Research No.49. (Goto M, Miller RW, eds.). Karger, Tokyo, Japan, 2001; pp.27-39.
16. Matsumoto T, Tsuchihashi Z, Ito C, Fujita K, Goto M, Furuichi Y. Genetic diagnosis of Werner's syndrome, a premature aging disease, by mutant allele specific amplification (MASA) and oligomer ligation assay (OLA). J Anti-Aging Med. 1998; 1:131-140.
17. Matsumoto T, Imamura O, Yamabe Y, Kuromutsu J, Tokutake Y, Shimamoto A, Suzuki N, Satoh Mi, Kitao S, Ichikawa K, Kataoka H, Sugawara K, Thomas W, Mason B, Tsuchihashi Z, Drayna D, Sugawara M, Sugimoto M, Furuichi Y, Goto M. Mutation and haplotype analyses of the Werner's syndrome gene based on its genomic structure:genetic epidemiology in the Japanese population. Hum Genet. 1997; 100:123-130.
18. Rothmund A. On cataract in conjunction with a characteristic skin degeneration. Graefes Arch Ophthal. 1868; 14:159-182.
19. Thomson MS. An hitherto undescribed familial disease. Br J Dermatol. 1923; 35:455-462.
20. Thannhauser SJ. Werner's syndrome (progeria of the adult) and Rothmund's syndrome; 2 types of closely related heredofamilial atrophic dermatoses; critical study with 5 new cases. Ann Intern Med. 1945; 23:559-626.
21. Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, Furuichi Y. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. Nat Genet. 1999; 22:82-84.
22. Ishida R. A case of cataract associated with scleroderma. Jpn J Ophthalmol. 1917; 21:1025-1032. (in Japanese)
23. Goto M, Miller RW, Ishikawa Y, Sugano H. Excess of rare cancers in Werner syndrome (Adult progeria). Cancer Epidemiol Biomarkers Prev. 1996; 5:239-246.
24. Oppenheimer BS, Kugel VH. Werner's syndrome: a heredofamilial disorder with scleroderma, bilateral juvenile cataract, precocious graying of hair and endocrine stigmatization. Trans Assoc Am Physicians. 1934; 49:358-370.
25. Oppenheimer BS, Kugel VH. Werner's syndrome, report of the first necropsy and findings in a new case. Am J Med Sci. 1941; 202:629-642.
26. Agatson SA, Gartner G. Precocious cataracts and scleroderma (Rothmund's syndrome: Werner's syndrome). Arch Ophthalmol. 1939; 21:492-496.
27. Hamada Y. Werner's syndrome: Report of a case with post-mortem findings. Jpn J Clin Dermatol Urol. 1966; 20:61-65. (in Japanese)
28. Koga M. Werner's syndrome associated with malignant melanoma. Jpn J Clin Dermatol Urol. 1968; 22:1160-1161. (in Japanese)
29. Goto M, Kindynis P, Resnick D, Sartoris DJ. Osteosclerosis of the phalanges in Werner syndrome. Radiology. 1989; 172:841-843.
30. Shiraki M, Aoki C, Goto M. Bone and calcium metabolism in Werner's syndrome. Endocr J. 1998; 45:505-512.
31. Endo K, Minamisaki T, yamashita H, Ozaki M, Yoshioka T, Yamashita H, Teshima R. A case of Werner syndrome associated with recurrent fibrosarcoma. J Jpn Orthop

Assoc. 2003; 77:S787.
32. Goto M, Horiuchi Y, Tanimoto K, Ishii T, Nakashima H. Werner's syndrome: Analysis of 15 cases with a review of the Japanese literature. J Am Geriatr Soc. 1978; 26:341-347.
33. Yokote K, Honjo S, Kobayashi K, Kawamura H, Mori S, Saito Y. Metabolic improvement and abdominal fat redistribution in Werner syndrome by pioglitazone. J Am Geriatr Soc. 2004; 52:1582-1583.
34. Martin GM, Gartler SM, Epstein CJ, Motulsky AJ. Diminished lifespan of cultured cells in Werner's syndrome. Fed Proc. 1965; 24:678.
35. Hatamochi A. Dermatological features and collagen metabolism in Werner syndrome. In: From premature gray hair to helicase-Werner syndrome:Implications for aging and cancer. Gann monograph on Cancer research No.49. (Goto M, Miller RW. Eds.). Karger, Tokyo, Japan. 2001; pp. 51-59.
36. Goto M, Yamabe Y, Shiratori M, Okada M, Kawabe K, Matsumoto T, Sugimoto M, Furuichi Y. Immunological diagnosis of Werner syndrome by down-regulated and truncated gene products. Hum Genet. 1999; 105:301-307.
37. Goto M, Kato Y. Hypercoagulable state indicates an additional risk factor for atherosclerosis in Werner's syndrome. Thromb Haemost. 1995; 73:576-578.
38. Yokote K, Hara K, Mori S, Kadowaki T, Saito Y, Goto M. Dysadiponectinemia in Werner syndrome and its recovery by treatment with pioglitazone. Diabetes Care. 2004; 27:2562-2563.
39. Hashizume H, Sato K, Takagi H, Kanda D, Kashihara T, Kiso S, Mori M. Werner syndrome as a possible cause of non-alcoholic steatohepatitis. J Clin Pathol. 2009; 62:1043-1045.
40. Goto M, Takeuchi F, Tanimoto K, Miyamoto T. Clinical, demographic, and genetic aspects of the Werner syndrome in Japan. In: Werner's syndrome and human aging. Advances in experimental medicine and biology. Vol. 190. (Salk D, Fujiwara Y, Martin GM. eds.). Plenum Press, New York, USA, 1985; pp. 245-261.
41. Takeuchi F, Kamatani N, Goto M, Matsuta K, Nishida Y, Nishioka K, Mikanagi K, Miyamoto T. Gout-like arthritis in patients with Werner's syndrome. Jpn J Rheumatol. 1987; 1:215-220.
42. Libby P. Inflammation in athrosclerosis. Nature. 2002; 420:868-874.
43. Mori S, Yokote K, Kondo Y, Murano S, Saito Y. Mild atherosclerosis in an autopsy case of Werner syndrome. Aging Dis. 1999; 12:742-745. (in Japanese)
44. Tokunaga M, Futami T, Wakamatsu E, Endo M, Yoshizawa Z. Werner's syndrome as "hyaluronuria". Clin Chim Acta. 1975; 62:89-92.
45. Goto M, Murata K. Urinary excretion of macromolecular acidic glycosaminoglycans in Werner's syndrome. Clin Chim Acta. 1978; 85:101-106.
46. Maeda H, Fujita H, Sakura Y, Miyazaki K, Goto M. A competitive enzyme-linked immunosorbent assay like method for the detection of urinary hyaluronan. Biosci Biotechnol Biochem. 1999; 63:892-895.
47. Tanabe M, Goto M. Elevation of serum hyaluronan level in Werner's syndrome. Gerontology. 2001; 47:77-81.
48. Laurent TC. Serum hyaluronan as a disease marker. Ann Med. 1996; 28:241-253.
49. Lindquvist U. The diurnal variation of serum hyaluronan in health and disease. Scand J Clin Lab Invest. 1988; 48:765-770.
50. Manicourt DH, Poilvache P, Nzeusseu A, van Egeren A, Devogelaer JP, Lenz ME, Thonar EJ. Serum levels of hyaluronan, antigenic keratin sulfate, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinases 1 change predictably in rheumatoid arthritis patients who have begun activity after a night of bed rest. Arthritis Rheum. 1999; 42:1861-1869.
51. Emlen W, Niebur J, Flanders G, Rutledge J. Measurement of serum hyaluronic acid in patients with rheumatoid arthritis: correlation with disease activity. J Rheumatol. 1996; 23:974-978.
52. Guechot J, Poupon RE, Giral P, Balkau B, Giboudeau J, Poupon R. Relationship between procollagen III aminoterminal propeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. J Hepatol. 1994; 20:388-393.
53. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panougia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Age Dev. 2007; 128:92-105.
54. Goto M. Inflammaging (inflammation+aging): A driving force for human aging based on an evolutionarily antagonistic pleiotrpy theory? BioSci Trends. 2008; 2:218-230.
55. Goto M, Nishioka K. Age-and sex-related changes of the lymphocyte subsets in healthy individuals: an analysis by two-dimensional flow cytometry. J Gerontol. 1989; 44:M51-56.
56. Goto M, Horiuchi Y, Okumura K, Tada T, Kawata M, Ohmori K. Immunological abnormalities of aging: an analysis of T lymphocyte subpopulations of Werner's syndrome. J Clin Invest. 1979; 64:695-699.
57. Goto M, Tanimoto K, Horiuchi Y, Kuwata T. Reduced natural killer cell activity of lumphocytes from patients with Werner's syndrome and recovery of its activity by purified human leukocyte interferon. Scand J Immunol. 1982; 15:389-397.
58. Goto M, Tanimoto K, Aotsuka A, Okawa M, Yokohari R. Age-related changes in auto- and natural antibody in the Werner's syndrome. Am J Med. 1982; 72:607-614.
59. Goto M, Tanimoto K, Miyamoto T. Immunological aspects of the Werner's syndrome. In: Werner's syndrome and human aging. Advances in experimental medicine and biology. Vol. 190. (Salk D, Fujiwara Y, Martin GM. eds.). Plenum Press, New York, USA, 1985; pp. 263-284.
60. Goto M. Immunosenescent features of human segmental progeroid syndrome: Werner's syndrome. Aging Immunol Infect Dis. 1992; 3:203-215.
61. Goto M, Sugimoto K, Hayashi S, Ogino T, Sugimoto M, Furuichi Y, Matsuura M, Ishikawa Y, Iwaki-Egawa S, Watanabe Y. Aging-associated inflammation in healthy Japanese individuals and the patients with Werner syndrome. Exp Gerontol. 2012; 47:936-939.
62. Sumi SM. Neuropathology of Werner syndrome. In: Werner's syndrome and human aging. Advances in experimental medicine and biology. Vol. 190. (Salk D, Fujiwara Y, Martin GM. eds.). Plenum Press, New York, USA, 1985; pp. 215-218.
63. Kuroda Y. Nervous system disorders in Werner syndrome. In: From premature gray hair to helicase-Werner syndrome:Implications for aging and cancer.

Gann Monograph on Cancer Research No.49. (Goto M, Miller RW, eds.). Karger, Tokyo, Japan, 2001; pp. 69-75.
64. Kakigi R, Endo C, Negishi R, Kohno H, Kuroda Y. Accelerated aging of the brain in Werner's syndrome. Neurology. 1992; 42:922-924.
65. Kawamura H, Tamura K, Yokote K, Mori S, Murano S, Saito K, Okada S, Kodama k, Satomi D. A case of Werner syndrome associated with Binswanger encephalopathy. Jpn J Geriatr. 1998; 35:578-579. (in Japanese)
66. Amagasa M, Yuda F. A case of Werner syndrome associated with multiple meningioma. Yamagata Saiseikann Ishi. 2005; 30:83-88. (in Japanese)
67. Mori H, Tomiyama T, Maeda N, Ozawa K, Wakasa K. Lack of amyloid plaque formation in the central nervous system of a patient with Werner syndrome. Neuropathology. 2003; 23:51-56.
68. Maekawa A, Fukudome K, Masuda Y, Sugawara H, Kubo T. A consideration on the background and pathogenesis of mood disorder in Werner syndrome. Shinshini. 2005; 45:236. (in Japanese)
69. Ishikawa Y, Sugano H, Matsumoto T, Furuichi Y, Miller RW, Goto M. Unusual features of thyroid carcinomas in Japanese patients with Werner syndrome and possible genotype-phenotype relations to cell type and race. Cancer. 1999; 85:1345-1352.
70. Iguchi H, Takayama M, Kusuki M, Sunami K, Nakamura A, Yamane H, Yamashita Y, Ohira M, Hirakawa K. A possible case of Werner syndrome presenting with multiple cancers. Acta Otolaryngol (Suppl). 2004; 554:67-70.
71. Satoh M, Matsumoto T, Imai M, Sugimoto M, Tsugane S, Furuichi Y, Goto M. Prevalence of Werner syndrome gene mutations in the Japanese population: A genetic epidemiological study. In: From premature gray hair to helicase-Werner syndrome: Implications for aging and cancer. Gann Monograph on Cancer Research No.49. (Goto M, Miller RW. eds.). Karger, Tokyo, Japan, 2001; pp. 19-25.
72. Satoh M, Imai M, Sugimoto M, Goto M, Furuichi Y. Prevalence of Werner's syndrome heterozygotes in Japan. Lancet. 1999; 353:1766.
73. Meguro S, Atsumi Y, Matsuoka K, Ishikawa Y, Sugimoto M, Goto M. Werner syndrome in a Korean man. Diabetes Diab Care. 2002; 25:1483-1484.
74. Martin GM, Sprague CR, Epstein CJ. Replicative life-span of cultivated human cells. Lab Invest. 1970; 23:86-92.
75. Ishikawa N, Nakamura K, Izumiyama-Shimomura N, Aida J, Ishii A, Goto M, Ishikawa Y, Asaka R, Matuura M, Hatamochi A, Kuroiwa M, Takubo K. Accelerated *in vivo* epidermal telomere loss in Werner syndrome. Aging. 2011; 3:417-429.
76. Labbe A, Lafleur VN, Patten DA, Robitaille GA, Garand C, Lamalice L, Lebel M, Richard DE. The Werner syndrome gene product (WRN): a repressor of hypoxia-inducible factor-1 activity. Exp Cell Res. 2012; 318:1620-1632.

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