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REVIEW ARTICLE

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What can we learn from Werner syndrome? A biased view from a rheumatologist

Abstract Werner syndrome (WS), caused by the mutation of the RecQ3 DNA helicase gene (loss of function), manifests scleroderma-like skin changes and juvenile cataracts in addition to a variety of clinical and biochemical aging phenotypes at an early stage of life, followed by death at an average age of 46 years. WS has been nominated as a top-ranking premature aging syndrome, or a human model of accelerated aging. Analyses of clinical and biological deterioration of body systems observed in WS may shed a unique light on the role of gene(s) in the pathogenesis of systemic sclerosis (SSc) and normal human aging.

Key words Autoantibody · Genetic instability · Helicase · Systemic sclerosis (SSc) · Werner syndrome (WS)

Introduction

Werner syndrome (WS; MIM#27770) is an autosomal recessive inherited disease.[1–4] Called an adult form of progeria, WS ranks the highest among normal human models of accelerated aging, or as a caricature of human aging, because of the similarities between normal human aging and WS patients.[1,3,5] Both the rarity of WS and the difficulty in differentiating it from autoimmune systemic sclerosis (SSc), especially outside of Japan, has probably led to an underestimation of its incidence.[3]

The rarity of the patients and the reduced growth potentials of their cells have forced me to minimize the study of WS although much attention has been paid to this unique syndrome since the mapping[6] and discovery of the WS gene (WRN:RecQ3 helicase).[7] I will review the clinical and biochemical aspects of WS in comparison with those of SSc, mainly based on our 30-year survey in Japan.

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History of WS research

Clarification of disease entity

The history of WS research begins with the publication of a doctoral dissectation by a German ophthalmologist, Otto Werner, in 1904.[8] He described several progeric manifestations observed in the patients, in addition to skin sclerosis and bilateral juvenile cataracts. He also assumed a genetic origin of the syndrome without any evidence of parental consanguinity, because the family of the patients came from a small Alpine valley village and the four siblings showed almost the same clinical signs and symptoms at a similar age. Thirty years later, the door to WS research was opened by two New York internists, Oppenheimer and Kugel, in 1934 and 1941, because they coined the name and published two papers on "Werner's syndrome."[9,10] The first reference of neoplasia in WS (fibroliposarcoma) was described by Agatson and Gartner in 1939.[11] A Boston internist, Thannhauser published a review article on WS in 1945,[12] and Seattle-based genetists, Epstein et al., released a landmark overview in 1966.[1] They suggested a possibility of an autosomal recessive mode of inheritance in this syndrome. Thus, the clinical entity of WS was established.

Genetics

In Japan, Ishida, an ophthalmologist in Kyoto University, reported the first Japanese case of WS in 1917.[13] Since then, case reporting of WS has accumulated in Japan. Autosomal recessive inheritance of WS was confirmed by the extensive analysis of 42 Japanese families, including 80 cases.[2] Seventy percent of the patients were offsprings of consanguineous marriage, mostly of first-cousin marriage. Also, clustering of patients in the same family has been frequently reported.

The phenotype of the *WRN* heterozygote has attracted strong interest, because Epstein et al.[1] suggested the existence of "formes frustes," or "abortive forms," and we

reported a relatively high frequency of cancer among the family members of patients with WS.[2] However, we still do not have definite results that heterozygous carriers show a part of the phenotypes which homozygous patients have.

Cellular aging

An important paper by Hayflick and Moorhead was published in 1961.[14] They reported the limited replicative capacity of the cultured skin fibroblasts and suggested a possible in vitro model of cellular aging. This paper led to the publications of Martin et al. showing that the replicative potential of skin fibroblasts from healthy individuals inversely correlated with the donor age from which the skin samples were obtained,[15] and also showing the strikingly diminished cultured life span of WS cells.[1,16] Although the growth potential of the stock fibroblasts is reduced, the primary culture cells from fresh biopsy samples of WS skin grow relatively well and the growth capacity is not as dramatically reduced as that reported (unpublished observation based on our own long experience).

Biochemical research

The clinical characteristics of WS were apparent in the connective tissue system: early onset of gray hair, alopecia, cataracts, bird-like face, skin sclerosis, short stature with stocky trunk, and extremely thin extremities (Cushing-like appearance). As a whole, these changes make WS patients elderly looking.[3] Tokunaga et al. detected excessive excretion of hyaluronan in the urine from WS patients, and coined the term hyaluronuria,[17] confirmed by us.[18–20] In addition, abnormal collagen metabolism was suggested.[21] However, studies of connective tissue metabolism in WS are still immature.[22]

Discovery of a gene

In 1981, we confirmed the genetics of WS: WS is transmitted as a single-gene, autosomal recessive trait.[2] With the help of rapid developments in the fields of molecular biology and human genetics, we started to map the WS gene using over 100 individuals. After a 3-years study, we mapped the *WRN* gene on the short arm of chromosome 8 (8p11–12).[6] This success promptly led to the cloning of *WRN* in 1996.[7] The DNA sequence of *WRN* indicated that the central portion of the predicted protein was related to the DNA helicase family.[23]

WRN belongs to RecQ3 helicase[24] with DNA helicase activity.[25] So far, 39 different mutations have been detected (unpublished observation and J. Oshima, 2002, personal communication). The details of the search for *WRN* were reported in a chapter of a recent monograph.[26]

Diagnosis of WS

Our diagnosis of WS was based on the presence of four of the five criteria for a patient under the age of 35 years.[2,3,27]

1. Consanguinity (mostly first-cousin marriage)
2. Characteristic bird-like appearance and body habitus (short stature, light body weight, and stocky trunk with spindly extremities)
3. Premature senescence (gray hair, alopecia, bilateral cataracts, hoarseness, osteoporosis, atherosclerosis, and malignancy)
4. Scleroderma-like skin changes (atrophic skin, skin sclerosis, skin ulcer, hyperkeratosis, hyper- or hypopigmentation, subcutaneous calcification, flat feet, and telangiectasia)
5. Endocrine-metabolic disorders (diabetes mellitus, hypogonadism, thyroid dysfunction, hyperuricemia, and hyperlipidemia)

Diagnosis of over 100 WS patients was further confirmed by the presence of the *WRN* mutation.[7,28–30] Some clinicians use the diagnostic criteria proposed by the International Registry of Werner Syndrome group, as follows, with permission (http://www.pathology.washington.edu/research/werner/registry/diagnostic.html; gmmartin@u.washington.edu):

Cardinal signs and symptoms (onset over 10 years old):
1. Cataracts (bilateral).
2. Characteristic dermatological pathology (tight skin, atrophic skin, pigmentary alterations, ulceration, hyperkeratosis, regional subcutaneous atrophy) and characteristic facies (“bird” facies)
3. Short stature
4. Parental consanguinity (third cousin or greater) or affected sibling
5. Premature greying and/or thinning of scalp hair
6. Positive 24-hour urinary hyaluronic acid test (when available)

Further signs and symptoms:
1. Diabetes mellitus
2. Hypogonadism (secondary sexual underdevelopment, diminished fertility, testicular or ovarian atrophy)
3. Osteoporosis
4. Osteosclerosis of distal phalanges of fingers and/or toes (x-ray diagnosis)
5. Soft tissue calcification
6. Evidence of premature atherosclerosis (e.g., history of myocardial infarction)
7. Mesenchymal neoplasms, rare neoplasms, or multiple neoplasms
8. Voice changes (high-pitched, squeaky, or hoarse voice)
9. Flat feet

Definite WS: All the cardinal signs and two others.
Probable WS: The first three cardinal signs and two others.
Possible WS: Either catarcts or dermatological alterations and any four others.
Exclusion: Onset of signs and symptoms before adolescence

(except stature, since current data on preadolescent growth patterns are inadequate.)

Because patients with WS show a wide variety of clinical manifestations, case reporting has been done from virtually all areas of medicine: internal medicine (diabetes mellitus and atherosclerosis), surgery (various cancers), neurosurgery (meningioma), psychiatry (schizophrenia), gynecology, urology (hypogonadism), ophthalmology (cataracts), dermatology (skin sclerosis and melanoma), otorhinolaryngology (hoarseness), plastic surgery (skin ulcer), radiology (subcutaneous calcification), and orthopedic surgery (leg gangrene and osteoporosis). Thus, the depth and width of the clinical descriptions about the patients have varied depending on the physician's speciality and interest. Also, information about the signs and symptoms observed in patients has often been subjective, retrospective, and subject to error. With the recent improvement of modern clinical laboratory techniques, a variety of clinical and laboratory examinations have been available to detect subtle physiologic changes. This may help in diagnosing a rare disease like WS more easily than before.

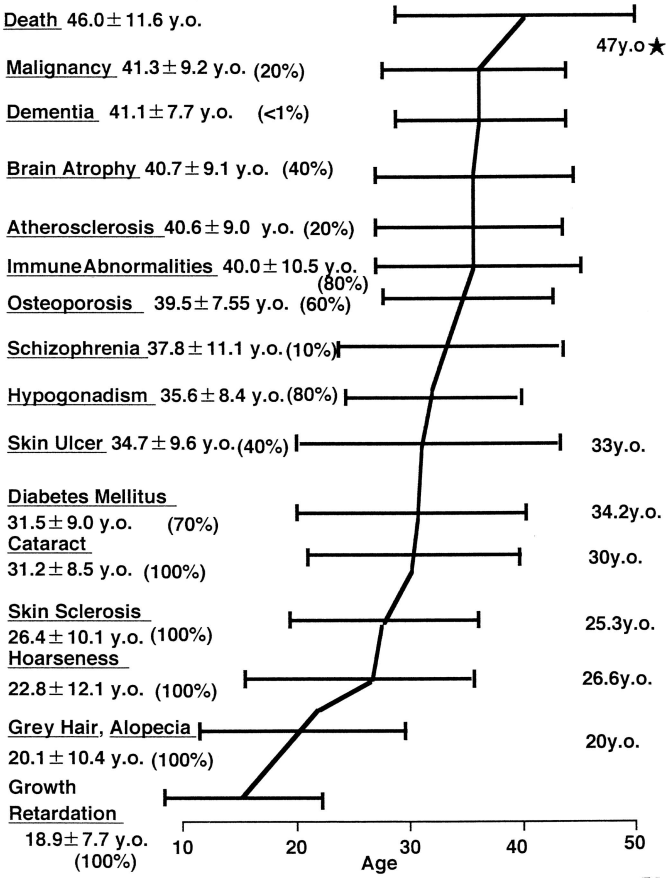

Fig. 1. Sequential appearance of clinical symptoms in Werner syndrome. The average age ±SD at which the typical clinical manifestations were observed in Werner syndrome is depicted. For comparison with the patients outside Japan (mostly Caucasians plus very few Negroes), the data from Epstein et al.[1] is indicated with a *star*

Clinical manifestations and natural history of WS

The sequentially appearing clinical hallmarks of patients with WS is shown in Fig. 1. After a relatively normal infancy, patients manifest a failure of the growth spurt at around age 18 years, followed by a hierarchical deterioration of the four major self-assembly (self-organization) body systems, as summarized in Table 1. Death occurs on average at the age of 46 years.[1,3] The major causes of death are neoplasia and myocardial infarction. Although data on normal aging are not enough to compare with those observed in WS, data on the normal development of self-assembly body systems from birth to late maturity with similar idea was published in 1930.[31]

Connective tissue system disorders

The parents of children with WS usually recognize the abnormality by their lack of the prepubertal growth spurt. All WS patients showed the following clinical signs and symptoms before the age of 32 years: characteristic habitus including slender extremities with stocky trunk (Cushingoid appearance); short stature (due to an early closure of bone end plate; range:122–161 cm) and light body weight (range: 19–52 kg); bird-like appearance with pinched nose and atrophic auricle; gray hair or alopecia; scleroderma-like skin changes including atrophic skin, subcutaneous tissue and muscle, hyper- or hypopigmentation, circumscribed hyperkeratosis, tight skin over the bones of the feet, and telangiectasia; bilateral cataracts; and a weak and high-pitched voice (hoarseness). Both skin ulcers and subcutaneous calcification, which are not usually associated with normal aging or autoimmune scleroderma, are found in 60%–80% of WS patients. Osteoporosis, either peripheral or vertebral, is observed in over 60% of patients.[32,33] These clinical manifestations may be mediated by connective tissue metabolites (adhesion molecules) such as gycosaminoglycans, fibronectin, and collagens (Table 2).

Endocrine-metabolic system disorders

Eighty percent of WS patients before the age of 36 years are recognized as having at least one of the following clinical signs and symptoms. Hypogonadism is observed in 80% of the patients, but about half of these patients show signs of hypogonadism after the age of 30 years and have offspring (secondary hypogonadism).[3] Noninsulin dependent diabetes mellitus of the specific type is associated with 70% of WS by the age of 36 years.[3] The mechanisms by which these clinical manifestations are induced are still unclear. However, in vitro experiments suggest an insulin-resistant mechanism: loss of signal transduction after its binding to normal insulin receptors.[37] Abnormality of the thyroid gland (~15%) includes Graves' disease, hypothyroidism, and thyroid carcinoma (the most frequent neoplasia in WS). Hyperuricemia is not usually associated with healthy eld-

Table 1. Hierarchical deterioration of body systems in Werner syndrome (WS) and systemic sclerosis (SSc)

Body system[a]	Clinical signs and symptoms	
	WS	SSc
Connective tissue (locomotive) (100%; 27.0 ± 10.4 years)	Scleroderma Mild fibrosis of internal organs Cataract Subcutaneous calcification Muscle atrophy Osteoporosis Gray hair or alopecia	Scleroderma Lung fibrosis Esophageal dilatation Calcinosis Muscle atrophy
Endocrine–metabolic (80%; 36.0 ± 8.7 years)	Hypogonadism Diabetes mellitus Thyroid dysfunction, cancer Hyperlipidemia, gout	Impotence
Immune (80%; 40.0 ± 10.5 years)	SLE Sjogren's syndrome	SLE Sjogren's syndrome Rheumatoid arthritis
Nervous (50%; 40.1 ± 9.8 years)	Brain atrophy, dementia Brain tumor Schizophrenia	Entrapment neuropathy
Mixed (50%; 40.8 ± 9.0 years)	Atherosclerosis Sarcoma	Raynaud phenomenon Lung cancer, renal crisis

SLE, systemic lupus erythematosus
[a] Relative percentage of the affected body system at the indicated age is shown in parentheses

Table 2. Extracellular matrix metabolism in Werner syndrome (WS) and systemic sclerosis (SSc)

	WS	SSc	Aging
Type I & III collagen expression and synthesis	↑	↑	↓
MMP-1 & MMP-3 expression and activity	↑	↓	↑
TIMP-1 expression	↓	?	↓
Fibronectin synthesis	↑	↑	↑
Hyaluronan synthesis	↑	↑	↑

↑, increased (expression, activity, and synthesis); ↓, decreased (expression, activity, and synthesis); MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproterinase

erly individuals. All types of hyperuricemia are found in WS: hyposecretion of uric acid, hyperproduction of uric acid, and a mixture of the two.[35] Hyperlipidemia characterized by hypertriglyceridemia is a biochemical hallmark of WS.[3,36]

Immune system disorders

The immune system is a very sensitive sytem to normal aging.[37,38] Before the age of 40 years, 80% of patients show signs of mild immune abnormality. A deficiency in a T-cell subset reactive against anti-brain-associated antigens was found in all the patients examined.[39,40] However, the number of patients studied was limited and the nature of the T-cell subset remains undefined. Decreased NK (natural killer) cell activity, which recovered after interferon stimulation, was observed in most of the patients examined.[41] Most of the patients had low titers of several autoantibodies, including anti-double-stranded DNA antibody, antinuclear antibody, and rheumatoid factor, as is usually observed in the healthy population over the age of 60 years.[39,40] 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 observed in some cases.[42] Interestingly, a small percentage of patients had autoimmune diseases, including Graves' disease, systemic lupus erythematosus, and Sogren's syndrome.[37] However, WS patients were not abnormally sensitive to bacterial or viral infection at any stage of their life, even though the third largest cause of death in WS is bacterial pneumonitis.

Nervous system disorders

WS patients were believed to have a relatively normal central nervous system.[43] However, with the recent advance of medical devices including computed tomography (CT) and magnetic resonance imaging (MRI), brain atrophy has been observed in 40% of WS patients, even before the age of 40 years.[3,44] At least three patients with WS have been diagnosed as having senile dementia, but not of the Alzheimer type by clinical determination and autopsy. It is interesting that 10% patients had schizophrenia at the age of 37 years, as shown in Fig. 1. Parkinsonism is closely associated with normal aging, but this is not the case with WS. Both farsightedness and hearing loss are major normal aging phenotypes. The percent of WS patients reported before 1970 showed signs of hearing loss as a result of otitis media infection. We do not know the patients' farsightedness status, probably because they had undergone a cataract operation.

Mixed system disorders

This type of self-assembly body system disorder is based on at least two of the earlier described body system disorders and the end results of normal aging. The two major causes of death in WS may be included in the category of atherosclerosis and malignancy. Atherosclerosis-related diseases, including myocardial infarction, angina pectoris, and hypertension, were observed in 50% of WS patients under the age of 40 years. These diseases consist mainly of decreased elasticity of blood vessels, probably because of the increased uptake of abnormal lipoproteins by macrophages. At least three systems (connective tissue, endocrine–metabolic, and immune systems) were involved in this process. The incidence of malignancy, especially of mesenchymal origin (sarcoma), is unusually high in WS (20%) before the age of 41 years.[45] The high incidence of malignancy in WS could be explained by its genetic instability based on the mutation of the RecQ3 helicase gene, which Salk described as variegated translocation mosaicism,[22] and a high frequency of somatic mutations.[46,47]

Epidemiology

Since the first description of WS by Otto Werner in 1904, case reporting has accumulated to approximately 1270 cases worldwide by early 2002.[3] About 80% of these cases are of Japanese ancestry. No patients have ever been reported in most African Countries or in most Asian countries, including mainland China and Korea, despite the historically frequent racial exchange with Japan. Some patients reported in the United States were of Japanese-American ancestry.[1] The frequency of WS is roughly estimated to be 1:100000 in Japan, whereas outside of Japan the frequency is 1:1000000–1:10000000. The only clustering of WS outside Japan occurs in Sardinia, Italy.[48] The discovery of the gene (*WRN*) enabled us to calculate a frequency of heterozygous carriers of 1:100 among the Japanese general population.[4] Mutation 4 (transversion of G to C at one-base upstream of 3370 in exon 25 resulted in a frame shift of codons 1078–1092), the most frequent mutation in Japanese patients, comprised 60% of the WS patients.[49,50] In Japan, several clustering areas have been noted: Miyagi Prefecture, South Kanto District, Ishikawa Prefecture, Hyogo Prefecture, and South Kyusyu District.[2,3,49,51]

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