Review

Werner's syndrome: From clinics to genetics

M. Goto

Makoto Goto, MD, PhD, Director of the Department of Rheumatology, Tokyo Metropolitan Otsuka Hospital, 2-8-1 Minami-Otsuka, Toshima-ku, Tokyo 170-0005, Japan.
E-mail: m.goto-o@ohtsuka-hospital.toshima.tokyo.jp

Clin Exp Rheumatol 2000; 18: 760-766.

Received on August 3, 2000; accepted on August 21, 2000.

Key words: Chromosomal instability, helicase, progeroid syndrome, systemic sclerosis, Werner's syndrome.

ABSTRACT

Werner's syndrome (WS), a representative progeroid syndrome with chromosomal instability caused by the mutation of RecQ type DNA/RNA helicase, manifests skin changes similar to those observed in systemic sclerosis (SSc). In addition, patients with WS show a variety of the signs and symptoms of normal ageing at an early stage of their life; gray hair, alopecia, muscle atrophy, osteoporosis, cataracts, hypogonadism, diabetes mellitus, hyperlipidemia, atherosclerosis, malignancy, brain atrophy, and senile dementia. Although no direct evidence has been presented linking RecQ type DNA/RNA helicase dysfunction with the occurrence of premature ageing symptoms in WS, WS may give us a unique model to analyze the skin changes and the mechanisms of fibrosis in SSc.

Introduction

Werner's syndrome (WS; MIM#27770), characterized by scleroderma and juvenile cataracts, is an autosomal recessively-inherited progeroid syndrome. WS has been recognized as a representative natural model of human ageing (1). Other progeroid syndromes or premature ageing syndromes include Cockayne's syndrome (MIM#21640, autosomal recessive), ataxia telangiectasia (Louis-Bar syndrome) (MIM#20890, autosomal recessive), Rothmund-Thomson syndrome (MIM#26840, autosomal recessive) and progeria (Hutchinson-Gilford progeria syndrome) (MIM#17667, autosomal dominant ?), in addition to chromosome 21-trisomy Down's syndrome.

Patients with these progeroid syndromes manifest relatively uniform signs and symptoms of a variety of elderly phenomenon (gray hair, alopecia, cataract, hoarseness, skin atrophy, hyper- or hypopigmentation, diabetes mellitus, osteoporosis, osteoarthritis, hypogonadism, brain atrophy, senile dementia, atherosclerosis and malignancy) at an early stage of their life (1-3). In addition to their early onset of age-related clinical manifestations and short life span, numerous *in vitro* experiments on progeroid syndrome cells (showing a diminished replicative life span of skin fibroblasts, accelerated telomere shortening, increased chromosomal instability and decreased immune function) and on body fluids (showing increased levels of serum fibronectin, serum and urinary hyaluronan, and serum autoantibodies) have suggested their striking similarity to normal ageing.

Despite the fact that much attention has been paid to these unique syndromes, the rarity of the patients and the reduced proliferative potentials of their cells have severely limited their study. Here I will review the clinical and genetical characteristics of a representative progeroid syndrome, Werner's syndrome, and discuss the differences between WS and autoimmune systemic sclerosis (scleroderma, SSc).

Historical background of Werner's syndrome research

Otto Werner, a medical student in the Ophthalmology Clinic at the Royal Albrecht University of Kiel, described four siblings with scleroderma and juvenile cataracts living in a small Alpine valley as his doctoral thesis in 1904 (4). He pointed out the possible genetic background of the condition and referred to the progeric features of this syndrome. Oppenheimer and Kugel drew attention to the disorder and coined the term "Werner's syndrome" (5, 6).

Extensive clinical and epidemiological studies by Epstein *et al.* (2) and Goto *et al.* (3, 7) confirmed the clinical entity of this syndrome and the presence of an autosomal recessive inheritance. A striking diminution in the growth potential of cultured skin fibroblasts from patients with WS was confirmed by Goldstein *et al.* (8) and Martin *et al.* (9), which suggested an acceleration of the replicative

lifespan of the cultured skin fibroblasts in this unique syndrome. In clinical studies Tokunaga *et al*. (10) and Goto *et al.* (11) reported an excessive excretion of urinary hyaluronan and named this finding "hyaluronuria". Hyaluronuria and the diminished cultured lifespan of skin fibroblasts represent the *in vitro* hallmarks of WS.

The clinical identification of WS as a genetic disorder transmitted by a single gene prompted us to proceed with a linkage analysis (12), followed by the successful cloning of the gene (WRN) in 1996 (13, 14). This gene encodes a type of RecQ DNA/RNA helicase (WRN). An extensive functional characterization of WRN has been conducted since then.

Clinical characteristics of Werner's syndrome

Since the first description of WS by Otto Werner in 1904, case reports have accumulated, to a total of 1,250 worldwide as of 1997 (3). About 80% of the patients are of Japanese origin, and no Oriental patients other than Japanese have ever been reported in the English literature. About 70% of the patients represent the offspring of marriages between first cousins.

As patients with WS show a wide variety of clinical manifestations, case reports have arrived from virtually all areas of medicine - from neurosurgery (meningioma) and psychiatry (schizophrenia) to ophthalmology (cataracts) and dermatology (skin sclerosis and melanoma). As a consequence, the depth and breadth of the clinical descriptions of the patients has varied depending upon the clinician's speciality and interest. In addition, information regarding the signs and symptoms observed in the patients has often been subjective, retrospective, and subject to error (2). However, with recent improvements in clinical laboratory techniques, a variety of clinical and laboratory examinations have become available to detect subtle physiologic changes. This review is mainly based on case reports published in Japan ever since WS was first described in 1917. The percentages of the respective clinical signs and symptoms should be taken into account as a somewhat rough estimate.

The diagnosis of WS in patients under the age of 35 is generally based on the presence of 4 out of the 5 following criteria (3, 12, 15): consanguinity; a characteristic bird-like or a mask-like appearance and body habitus (short stature with a stocky trunk and very thin extremities; Cushingoid appearance); premature senescence (gray hair, alopecia, cataracts, hoarseness, osteoporosis, arteriosclerosis, and malignancy); scleroderma-like skin changes (atrophic skin, skin sclerosis, skin ulcer, hyperkeratosis, hyper- or hypopigmentation, subcutaneous calcification, and telangiectasia); and endocrine-metabolic disorders (diabetes mellitus, hypogonadism, thyroid dysfunction, hyperuricemia, and hyperlipidemia). In addition, in more than two studies over 100 WS patients were further examined for the presence of the WRN mutation (12, 14, 16), hyaluronuria (10, 11,17), decreased replicative lifespan of skin fibroblasts, autoantibodies (18, 19, 20), and decreased natural killer cell activity (21).

The hierarchical deterioration in the clinical hallmarks of the patient with WS is shown in Figure 1. After a relatively normal infancy, by the age of 18 they have failed to manifest the normal pre-

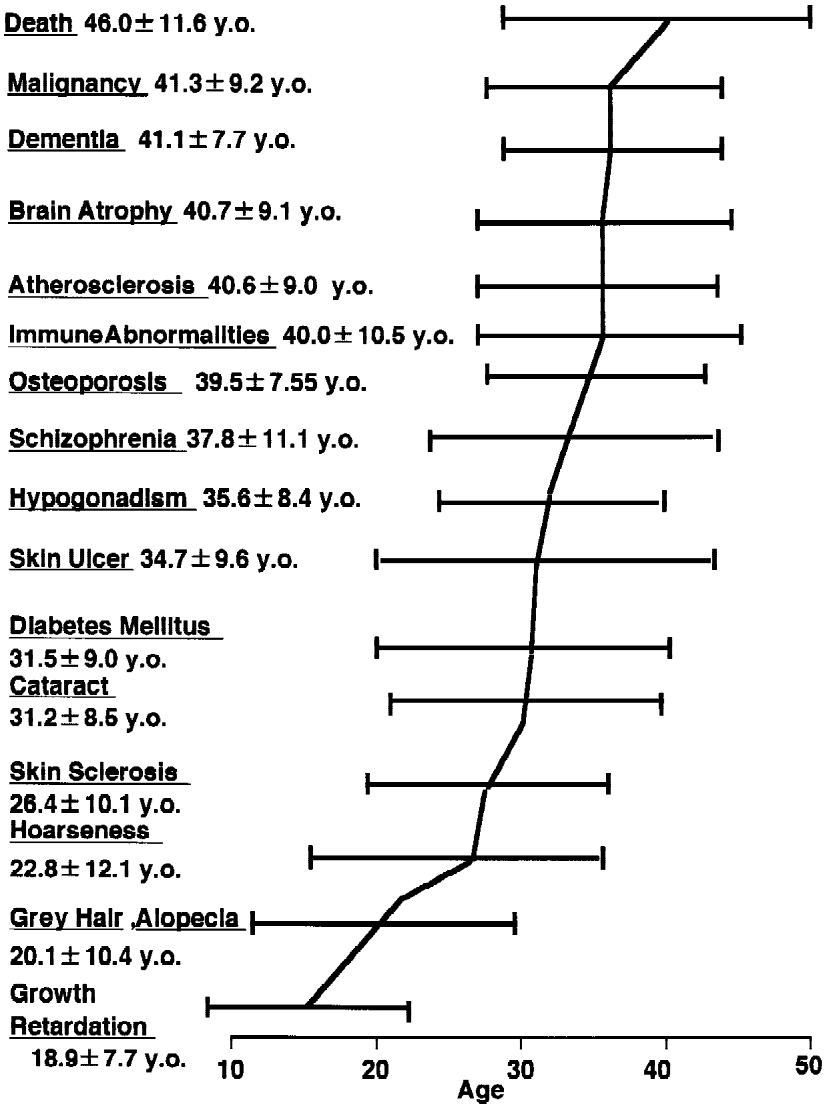

Fig. 1. Sequential appearance of the typical clinical symptoms of Werner's syndrome. The average age ± SE at which each manifestation is observed is indicated.

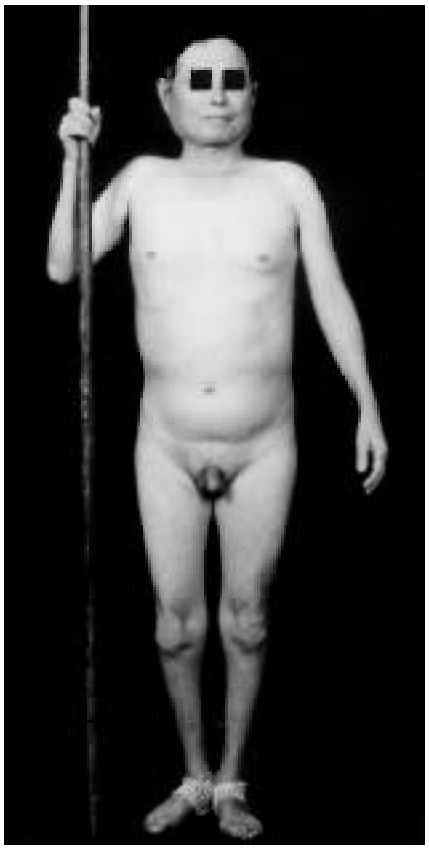

Fig. 2. The typical Cushingoid appearance in a 42-year-old male patient with Werner's syndrome: a stocky trunk with thin extremities. The patient also had testicular atrophy with gynecomastia, and intractable skin ulcers on both heels. He was totally bald and in this picture is shown wearing a wig. He died of renal failure due to atherosclerosis of the renal arteries at the age of 43.

Fig. 3. The "bird-like" or "mask-like" face typical of Werner's syndrome in the same patient shown at different ages, as indicated. He had the typical pinched nose with adhesive ears at age 39.

pubertal growth spurt. This is followed by failures in 4 major body systems, appearing sequentially as summarized below. Among the major clinical signs and symptoms observed in this unique syndrome, the characteristic habitus and stature, scleroderma, gray hair, osteoporosis (22, 23), hyaluronuria (10, 11, 17) and cataracts may be classified as connective tissue system-based manifestations (incidence: 100% at age 27 ± 10.4 y.o.). Diabetes mellitus, hypogonadism, thyroid dysfunction, hyperlipidemia (24), and hyperuricemia (25) may be classified as endocrine-metabolic system-based manifestations (incidence: 80% at age 36 ± 8.7 y.o.). Depressed NK activity, increased autoantibody production, and autoimmune diseases (18,19,20) can be classified as immune system-based manifestations (incidence: 80% at age 40 ± 10.5 y.o.), and brain atrophy (26), senile dementia (27) and schizophrenia as nervous system-based clinical manifestations (incidence: 50% at age 40.1 ± 9.8 y.o.), respectively. Thereafter, disorders involving more than one system ensue, including atherosclerosis-related disorders at age 40, and a variety of malignancies at an average age of 41 followed by death at an average age of 46 years (2, 3). Malignancy and atherosclerosis-related disorders (myocardial infarction and cerebral thrombosis) constitute the two major causes of death in WS.

Connective tissue (locomotive) system disorders

WS patients show a wide variety of the senescent phenotypes observed in normal ageing and also the skin changes characteristic of SSc. Patients with WS are usually recognized by their lack of a teenage growth spurt; short stature (141.5 ± 21.8 cm) and low body weight (35.7 ± 17.9 kg). Basically, all WS patients show the following clinical signs and symptoms before the age of 32: characteristic habitus including slender extremities with a stocky trunk, short stature and low body weight (Cushingoid appearance) (Fig. 2); a bird-like or mask-like appearance (Fig. 3); gray hair or alopecia; scleroderma-like skin changes including atrophic skin, subcutaneous tissue and

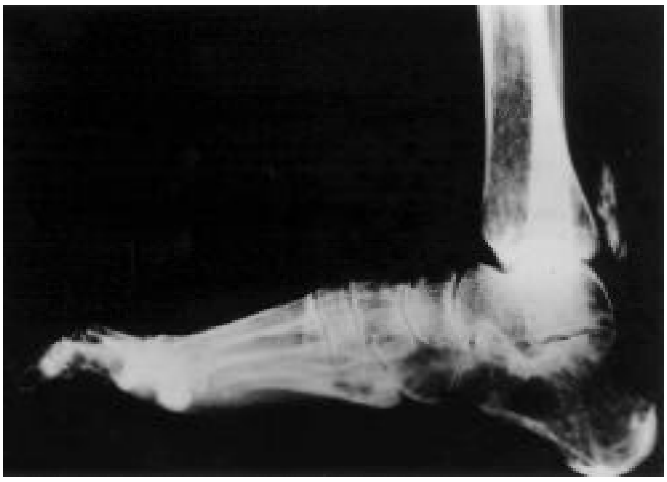

Fig. 4. Subcutaneous calcifications along the Achilles tendon characteristic of WS, in a 53-year-old male patient. Osteoporosis is also present.

Table I. Hierarchical deterioration of body systems in Werner's syndrome (WS) and systemic sclerosis (SSc). For each body system, the percentage of WS patients affected at the indicated age is shown between parentheses. The typical symptoms of this body system involvement are listed.

Body systems	Clinical signs and symptoms	
	Werner's syndrome	Systemic sclerosis
1. Connective tissue (locomotive) (100%; 27.0 ± 10.4 y.o.)	Scleroderma Mild fibrosis of internal organs Cataract Subcutaneous calcification Muscle atrophy Osteoporosis Gray hair or alopecia	Scleroderma Lung fibrosis Esophageal dilatation Calcinosis Muscle atrophy
2. Endocrine-metabolic (80%; 36.0 ± 8.7 y.o.)	Hypogonadism Diabetes mellitus Thyroid dysfunction, cancer Hyperlipidemia, gout	Impotence
3. Immune (80%; 40.0 ± 10.5 y.o)	Systemic lupus erythematosus Sjögren's syndrome	Systemic lupus erythematosus Sjögren's syndrome Rheumatoid arthritis
4. Nervous (50%; 40.1 ± 9.8 y.o.)	Brain atrophy, dementia Brain tumor Schizophrenia	Entrapment neuropathy
5. Mixed (50%;40.8 ± 9.0 y.o.)	Atherosclerosis Sarcoma	Raynaud's phenomenon Lung cancer

Table II. Extracellular matrix metabolism in Werner's syndrome (WS) and scleroderma (SSc). For comparison, the changes in extracellular matrix metabolism in the normal ageing process are included.

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

muscle, skin hyper-or hypopigmentation, circumscribed hyperkeratosis, tight skin over the bones of the feet and telangiectasia; bilateral cataracts; and a weak, high-pitched voice (hoarseness). Both skin ulcers and subcutaneous calcification (Fig. 4), which are associated with a specific form of SSc (CREST), but not usually associated with normal ageing, are characteristically found in 60-80% of WS patients. Osteoporosis either of the limbs or vertebra are observed in over 60% of patients (22, 23).

Because of the similar extracellular matrix metabolism and the resultant similarity in the skin changes seen in WS and SSc, patients with WS are frequently misdiagnosed as having SSc in Japan. For comparison, the major similarities and differences between SSc and WS are summarized in Table I. In addition, however, there are many minor differences. In the extracellular matrix metabolism, the production of collagens type I and III, fibronectin and hyaluronan in the skin are increased in both conditions. However, in contrast to the increased activity of both matrix metalloproteinases-1 and -3 in the cultured skin fibroblasts from WS patients and normally ageing subjects, those activities are decreased in SSc (Table II) (28).

Endocrine-metabolic system disorders

Before the age of 36, 80% of WS patients are recognized as having at least one of the clinical signs and symptoms of an endocrine and metabolic system disorder. Hypogonadism is observed in 80% of patients, but about half of these show signs of hypogonadism only after the age of 30 and succeed in having offspring (secondary hypogonadism) (29). Non-insulin dependent diabetes mellitus is present in 70% of WS patients by the age of 36 (3, 29). The mechanisms by which these clinical manifestations are induced remain unclear. However, *in vitro* experiments have suggested the presence of an insulin-resistant mechanism, i.e. the loss of signal transduction after the binding of normal insulin to normal insulin receptors (30). Thyroid dysfunction, either hyper- or hypo-functioning, is observed in 15% of WS patients.

In SSc fibrosis invades the thyroid gland in 15% of patients, usually without any obvious functional abnormalities (31). Impotence, probably caused by abnormal penile vascular functioning, has been reported in an early stage of SSc (32). Hyperuricemia is not usually associated with either healthy elderly individuals or SSc. All types of hyperuricemia are found in WS, however: hyposecretion of uric acid, hyperproduction of uric acid, and mixed form involving the two conditions (25). Hyperlipidemia type IIb or type IV, characterized by intractable hypertriglyceridemia and hypercholesterolemia, is a biochemical hallmark of WS (3, 24).

Immune system disorders

The immune system is very sensitive to the normal ageing process (20, 33). Before the age of 40, 80% of WS patients show some signs of immune abnormality. In one study, deficiency in the T cell subset that is reactive against anti-brain associated antigens was found in all the patients examined (18). However, the exact nature of the T cell subset remains undefined. Decreased NK cell activity, which was restored by interferon treatment, has been observed in most WS patients and also in SSc patients (21). Most patients have low titers of several autoantibodies including anti-DNA antibody, antinuclear antibody and rheumatoid factor, as is usually observed in the healthy population over 60 y.o. (18, 20). However, the autoantibodies characteristic of SSc, such as Scl 70 (anti-topoisomerase) and anti-centromere antibodies, have never been detected in WS.

Interestingly, a small percentage of patients have autoimmune diseases including Möbius' syndrome, systemic lupus erythematosus (SLE), or Sjögren's syndrome (SS). SSc, too, is frequently associated with other types of autoimmune diseases including SLE, SS and rheumatoid arthritis. However, WS patients are not abnormally sensitive to bacterial or viral infection at any stage of their life (20).

Nervous system disorders

It was once believed that WS patients had a relatively normal central nervous system (34). However, with recent developments in sophisticated medical devices including CT (computed tomography) and MRI (magnetic resonance imaging), brain (especially cortical brain) atrophy has been observed in 40% of WS patients even before the age of 40. A small percentage of patients with WS have been diagnosed as having senile dementia (27), but not of the Alzheimer type, on the basis of clinical examination or autopsy. It is interesting to note that 10% of patients had schizophrenia by the age of 37, as shown in Figure 1.

In general SSc spares the central nervous system. However, entrapment neuropathies, including carpal tunnel syndrome and facial nerve palsies, are sometimes encountered (35).

Mixed system disorders

This type of system disorder is characterized by involvement of at least two of the body systems described above.

Atherosclerosis and malignancy, the major causes of death in WS, may be included in this category. Atherosclerosis and atherosclerosis-related diseases, including myocardial infarction, angina pectoris, cerebral hemorrhage, cerebral thrombosis and hypertension are observed in 50% of WS patients before the age of 40. This consists mainly of decreased elasticity of the blood vessels due to the increased uptake of abnormal lipoproteins by macrophages. At least 3 systems (the connective tissue, endocrine-metabolic, and immune systems) are included in this process.

The incidence of malignancy, especially of mesenchymal origin (sarcoma), is particularly high in WS (20%) before the age of 41, as was extensively reviewed by M. Goto (36). The high incidence of malignancy in WS could be partially explained by the chromosomal instability of WRN, which D. Salk summarized as variegated translocation mosaicism (37), and by a high frequency of somatic mutations (38, 39). In patients with SSc a high risk of lung cancer (alveolar cell carcinoma) superimposed on long-standing interstitial pulmonary fibrosis has been observed (40).

Both Raynaud's phenomenon and renal crisis similar to that seen in SSc may be included in the mixed system disorder category.

Demographics and genetics

A total of more than 1,250 patients with WS have so far been reported from all over the world (3). Japan has the largest number of patients (over 950), followed by the U.S. (69), and Germany (51). An analysis of more than 200 Japanese families and Caucasian families have suggested an autosomal recessive inheritance of this syndrome (2, 7). After confirmation of the hypothesis that WS is transmitted by a single gene, a search for the gene itself was started at the beginning of 1989.

By linkage analysis using polymorphic (CA)n repeats, the gene for WS (WRN) was mapped to the short arm of chromosome 8 (8p12), as indicated in Figure 5 (12). After this discovery the gene, which encodes RecQ type DNA/RNA helicase, was cloned in 1996 (13, 14). A total of 19 different mutations have been reported (16). RecQ type helicase is a type of helicase with a high homology to *E. coli* helicase:*rec.* It is an enzyme which unwinds the helical chain of double-stranded DNA/RNA in association with other enzymes during any event of DNA/RNA metabolism in the nucleus: repair, replication, recombination, and transcription (41).

The RecQ type WRN helicase shows typical helicase activity (42), but in WS patients the protein is truncated and thus its helicase activity is defective (43). Over 500 different helicases have been reported in organisms ranging from viruses to man; humans have 5 different RecQ type helicases. Three different RecQ type helicase deficiency disorders have been identified so far: Bloom's syndrome (BLM), Werner's syndrome (WRN) and Rothmund-Thomson syndrome (RecQL4) (44). All of these "helicase diseases" are cancer-prone syndromes with chromosomal instability.

There may be at least two reasons why Japan has the largest number of WS patients. First, the frequency of consanguineous marriages (first cousin marriages), especially in the mountainous areas of Japan, is high. Second, the frequency of heterozygous healthy carriers of WRN in Japan is extremely high – roughly 1:100 (45) – a ratio which may be more than 100 times higher than in the rest of the world.

In a separate study no WRN mutation was detected in 30 Japanese patients with SSc.

Conclusion

Werner's syndrome, which is caused by a mutation of the RecQ type DNA/RNA helicase (WRN), manifests skin changes similar to those in SSc. The skin sclerosis seen in SSc has been proposed to be the result of an unknown autoimmune mechanism in the extracellular matrix metabolism. Although no direct relationship between the helicase involved and the extracellular matrix metabolism has been found, WS may provide us with a unique tool to probe the abnormal extracellular matrix metabolism in SSc.

References

1. MARTIN GM: Genetic syndromes in man with potential relevance to the pathobiology of aging. *Birth Defects* 1978; 14: 5-39.
2. EPSTEIN CJ, MARTIN GM, SCHULTZ AL, MOTULSKY AG: Werner's syndrome. A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine* 1966; 45: 177-221.
3. GOTO M: Hierarchical deterioration of body systems in Werner's syndrome: Implications for normal ageing. *Mech Age Dev* 1997; 98: 239-54.
4. WERNER O: On cataract in conjunction with scleroderma (doctoral dissertation). Kiel University, Schmidt and Klaunig, Kiel, 1904.
5. OPPENHEIMER BS, KUGEL VH: Werner's syndrome - A heredo-familial disorder with scleroderma, bilateral juvenile cataract, precocious graying of hair and endocrine stigmatization. *Trans Ass Amer Physicians* 1934; 49: 358-70.
6. OPPENHEIMER BS, KUGEL VH: Werner's syndrome, report of the first necropsy and of findings in a new case. *Am J Med Sci* 1941; 202: 629-42.
7. 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.
8. GOLDSTEIN S, LITTEFIELD JW, SOELDER JS: Diabetes mellitus and ageing: Diminished plating efficiency of cultured human fibroblasts. *Proc Natl Acad Sci USA* 1969; 64: 155-60.
9. MARTIN GM, SPRAGUE CR, EPATEIN CJ: Replicative life-span of cultivated human cells. *Lab Invest* 1970; 23: 86-92.
10. TOKUNAGA M, FUTAMI T, WAKAMATSU E, ENDO M, YOSHIZAWA Z: Werner's syndrome as 'hyaluronuria'. *Clin Chim Acta* 1975; 62: 89-92.
11. GOTO M, MURATA K: Urinary excretion of macromolecular acidic glycosaminoglycans in Werner's syndrome. *Clin Chim Acta* 1978; 85: 101-6.
12. 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: 735-8.
13. YU C-E, OSHIMA J, FU Y-H *et al.*: Positional cloning of the Werner's syndrome gene. *Science* 1996; 272: 258-62.
14. GOTO M, IMAMURA O, KUROMITSU J *et al.*: Analysis of helicase gene mutations in Japanese Werner's syndrome patients. *Hum Genet* 1997; 99: 191-3.
15. 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 Geriatrics Soc* 1978; 26: 341-7.
16. MATSUMOTO T, IMAMURA O, YAMABE Y *et al.*: 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: 123-30.
17. TANABE M, MAEDA M, MIYAZAKI K, GOTO M: Elevation of serum hyaluronan level in Werner's syndrome. *Gerontol* (in press).
18. 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: 695-9.
19. GOTO M, TANIMOTO K, AOTSUKA S, OKAWA M, YOKOHARI R: Age-related changes in auto- and natural antibody in Werner's syndrome. *Am J Med* 1982; 72: 607-14.
20. GOTO M: Immunosenescent features of human segmental progeroid syndrome: Werner's syndrome. *Aging Immunol Infect Dis* 1992; 3: 203-15.
21. GOTO M, TANIMOTO K, HORIUCHI Y, KUWATA T: Reduced natural killer cell activity of lymphocytes from patients with Werner's syndrome and recovery of its activity by purified human leukocyte interferon. *Scand J Immunol* 1982; 15: 389-97.
22. GOTO M, KINDYNIS P, RESNICK D, SARTORIS DJ: Osteosclerosis of the phalanges in Werner's syndrome. *Radiology* 1989; 172: 841-3.
23. SHIRAKI M, AOKI C, GOTO M: Bone and calcium metabolism in Werner's syndrome. *Endocrine J* 1998; 45: 505-12.
24. GOTO M, KATO Y: Hypercoagulable state indicates an additional risk factor for atherosclerosis in Werner's syndrome. *Thromb Haemost* 1995; 73: 576-8.
25. TAKEUCHI F, KAMATANI N, GOTO M *et al.*: Gout-like arthritis in patients with Werner's syndrome. *Jap J Rheumatol* 1987; 1: 214-20.
26. KAKIGI R, ENDO C, NESHIGE R, KOHNO H, KURODA Y: Accelerated aging of the brain in Werner's syndrome. *Neurology* 1992; 42: 922-4.
27. KURODA Y: Nervous system disorders in Werner's syndrome. *In* GOTO M and MILLER RW (Eds.): *From Premature Gray Hair to Helicase: Werner's Syndrome: Implications for Ageing and Cancer*. Monograph on Cancer Research No. 44, Japan Scientific Societies Press, Tokyo, 2000; in press.
28. HATAMOCHI A: Dermatological features and collagen metabolism in Werner's syndrome. *In* GOTO M and MILLER RW (Eds.): *From Premature Gray Hair to Helicase: Werner's Syndrome: Implications for Ageing and Cancer*.

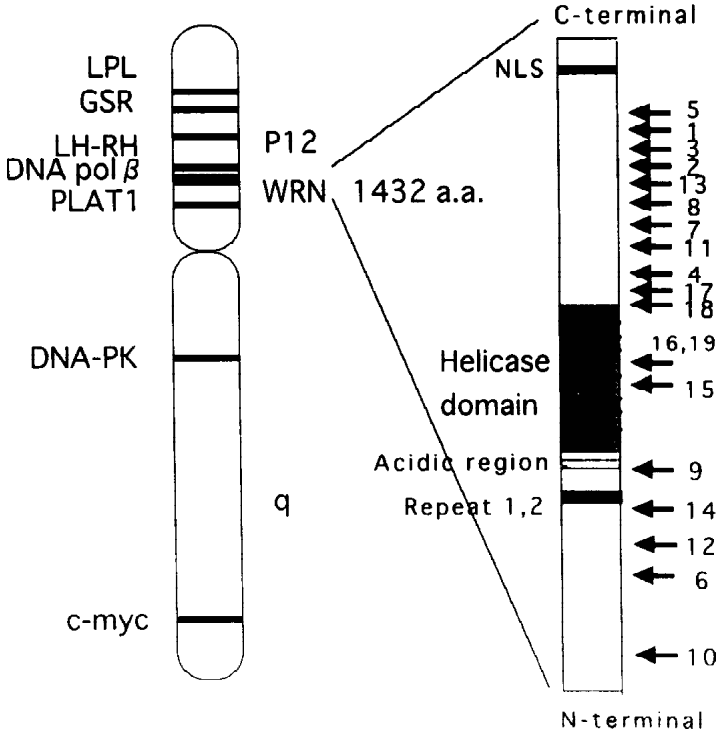

Fig. 5. WRN gene location on the chromosome 8.

Monograph on Cancer Research No. 44, Japan Scientific Societies Press, Tokyo, 2000; in press.
29. GOTO M, TAKEUCHI F, TANIMOTO K, MIYAMOTO T: Clinical, demographic and genetic aspects of the Werner's syndrome in Japan. *In* SALK D, FUJIWARA Y & MARTIN GM (Eds.): *Werner's Syndrome and Human Aging*, Plenum Press, New York and London, 1985: 245-61.
30. KAKEHI T, KUZUYA H, YOSHIMURA Y *et al.*: Binding and tyrosine kinase activities of the insulin receptor on Epstein-Barr virus transformed lymphocytes from patients with Werner's syndrome. *J Gerontol* 1988; 43: M40-45.
31. GORDON MB, KLEIN I, DEKKER A, RODNAN GP, MEDSGER TA JR: Thyroid disease in progressive systemic sclerosis: Increased frequency of glandular fibrosis and hypothyroidism. *Ann Intern Med* 1981; 95: 431-4.
32. NOWLIN NS, BRICK JE, WEAVER DJ *et al.*: Impotence in scleroderma. *Ann Intern Med* 1986; 104: 796-800.
33. GOTO M, NISHIOKA K: Age- and sex-related changes of the lymphocyte subsets in healthy individuals: An anlysis by two-dimensional flow cytometry. *J Gerontol* 1989; 44: M51-56.
34. SUMI SM: Neuropathology of Werner's syndrome. *In* SALK D, FUJIWARA Y and MARTIN GM (Eds.): *Werner's Syndrome and Human Aging*, Plenum Press, New York and London, 1985: 215-8.
35. KAHL LE, MEDSGER TA JR, KLEIN I, RODNAN GP: Prospective evaluation of thyroid function in patients with progressive systemic sclerosis. *Arthritis Rheum* 1983; 26: S62.
36. GOTO M, MILLER RW, ISHIKAWA Y, SUGANO H: Excess of rare cancers in Werner's syndrome (adult progeria). *Cancer Epidemiol Biomarker Prevention* 1996; 5: 239-46.
37. SALK D: Werner's syndrome: A review of recent research with an analysis of connective tissue metabolism, growth control of cultured cells, and chromosomal aberrations. *Hum Genet* 1982; 62:1-15.
38. FUKUCHI K, TANAKA K, KUMAHARA Y *et al.*: Increased frequency of 6-thioguanine-resistant peripheral blood lymphocytes in Werner's syndrome patients. *Hum Genet* 1990; 84: 249-52.
39. KYOIZUMI S, SEYAMA T, HATAMOCHI A, GOTO M: *In vivo* somatic mutations in Werner's syndrome. *Hum Genet* 1998; 103: 405-10.
40. CIPOLETTI JF, BUCKINGHAM RB, BARNES EL *et al.*: Sjögren's syndrome in progressive systemic sclerosis. *Ann Intern Med* 1977; 87: 535-40.
41. GORBALENYA AE, KOONIN EV, DONCHENKO AP, BLINOV VM: Two related superfamilies of putative helicases involved in replication, recombination, repair, and expression of DNA and RNA genomes. *Nucl Acids Res* 1989; 17: 258-62.
42. SUZUKI N, SHIRATORI M, GOTO M, FURUICHI Y: Werner's syndrome helicase contains a 5' —> 3' exnuclease activity that digests DNA strands in DNA/DNA and RNA/DNA duplexes dependent on unwinding. *Nucl Acids Res* 1999; 27: 2361-88.
43. MATSUMOTO T, SHIMAMOTO A, GOTO M, FURUICHI Y: Impaired nuclear localization of defective DNA helicases in Werner's syndrome. *Nature Genet* 1997; 16: 335-6.
44. KITAO S, SHIMAMOTO A, GOTO M *et al.*: Mutations of RecQL4 cause a subset of Rothmund-Thomson syndrome. *Nature Genet* 1999; 22: 82-4.
45. SATOH M, IMAI M, SUGIMOTO M, GOTO M, FURUICHI Y: Prevalence of Werner's syndrome heterozygotes in Japan. *Lancet* 1999; 353: 1766.