Brief Report

Syndrome-causing mutations in Werner syndrome

Makoto Goto*

Division of Anti-Ageing and Longevity Sciences, Department of Clinical Engineering, Faculty of BioMedical Engineering, Toin University of Yokohama, 1614 Kurogane-Cho, Aoba-ku, Yokohama, Japan.

Summary **Complete loss of function in the WRN: RecQ3 DNA/RNA helicase gene causes Werner Syndrome (WS). WS patients with genetic instability manifest an early onset of age-related diseases including diabetes mellitus (DM), osteoporosis, atherosclerosis, and malignancy as well as early death. In 1,420 patients, WS was reported to be associated with chromosomal abnormality syndrome and other genetic diseases including Klinefelter syndrome in 2 patients, retinitis pigmentosa in 3, Wilson's disease in 1, xeroderma pigmentosum in 3, and porokeratosis Mibelli in 1. These clinical findings may support the concept of genetic instability in WS.**

Keywords: Aging, Genetic instability, Mutation, Werner syndrome, Xeroderma pigmentosum

1. Introduction

Half a century ago, Dr. Alex Comfort encouraged medical researchers to look for evidence of chromosomal abnormalities in many forms of constitutional disorders, the most important of which was Werner syndrome (WS: MIM#27770) (*1*). However, his theory faltered since normal chromosomes were found in WS (*2-5*). WS, the gene located at chromosome 8p11-12 (*6*) and caused by a recessively mutated WRN, is characterized by a variety of clinical manifestations mimicking features of advanced aging and thus may represent a typical progeroid syndrome (*7*). *Wrn* is a member of the RecQ helicase gene family (*RecQ3*) and may interact with a variety of DNA/RNA metabolism enzymes during repair, transcription, translation, recombination, replication, and chromosome segregation in the nucleus (*8*). Thus, actively proliferating cells may be affected by WRN dysfunction. Theories on the function of RecQ helicases and *in vitro* studies using WS fibroblasts and peripheral blood cells have suggested genomic instability in WS cells (*8-12*). Patients with WS do not usually have apparent abnormalities before their teenage growth spurt, but they typically display hierarchical deterioration of a variety of connective tissue systems resulting in physical symptoms such as gray hair, alopecia, skin atrophy, skin sclerosis, skin hyper/hypo-pigmentation, vocal cord atrophy, osteoporosis, sarcopenia, bilateral cataracts, metastatic subcutaneous calcification, and atherosclerosis. Connective or supportive tissue may be a source of malignancies (and particularly sarcomas), and adversely affected systems include the endocrine system, resulting in type II diabetes mellitus (DM), hypogonadism, and thyroid disorders, and the metabolic system, resulting in hyperlipidaemia, hyperuricemia and hyaluronuria. Systems affected to a lesser degree include the immune system, resulting in excessive auto-antibody production, impaired cytokine response, and natural killer cell activity, and the nervous system, resulting in cognitive disorders and brain atrophy (*5,7,13*). Death due to malignancy or atherosclerosis-related conditions such as myocardial infarction typically occurs in the late 40s (*13-15*). In addition, *in vivo* mutation as may be associated with genetic instability of WS cells may induce other genetic diseases.

Since the first description of WS by the German family physician Otto Werner in 1904 (*16*), 1,420 cases of WS have been reported in total worldwide (*17*). Interestingly, 75% of WS patients are of Japanese

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

descent (*14*), which is probably due to the relatively high frequency of consanguineous marriage in rural areas and an extremely high prevalence (1:100) of heterozygosity in the general Japanese population (*18,19*). Approximately 10 WS cases per year are regularly documented in Japan, while only 3.3 patients per year are reported outside the country. Adjusting roughly for population size, the frequency of WS in Japan is some 150-fold greater than in the rest of the world (Japan's population between 1966 and 2004 was about 113,000,000 with respect to a world population of about 5,000,000,000; that said, case reports are highly encouraged in Japan in comparison to some parts of the world).

Since the cloning of the WRN: RecQ3 helicase gene, the search for mutation-causing mutations in WS has been a matter of scientific/clinical interest (*20*). The current study looked for chromosomal abnormalities and genetic diseases associated with WS in WS patients.

2. Materials and Methods

The first case of WS in Japan was reported in 1917 (*21*). Here, the clinical manifestations of WS as described in all papers published between 1917 and 2004 were analyzed. WS publications were selected through a citation index (Igaku-Chuo-Zasshi) and bibliographies of each report were extensively examined for additional references. For comparison of Japanese and foreign patients with WS, searches were performed using PubMed. Care was taken to thoroughly identify patient family details, personal histories, authors, institutions, and demographic characteristics to avoid the inclusion of duplicate patient data.

As most patients were diagnosed clinically with WS, diagnoses given by the original authors were carefully re-evaluated based on the presence of the following phenotypes: unusual body habitus, bilateral cataracts, skin sclerosis, painful corns, sarcopenia, metastatic subcutaneous calcification, skin ulcers, DM, and hyperlipidaemia (*7,13,14*).

3. Results and Discussion

A total of 1,070 cases documented in 500 Japanese articles published between 1917 and 2004 were included in the analysis. Outside Japan, 350 cases of WS have been reported. As shown in Table 1, 1 case of WS associated with Klinefelter syndrome was diagnosed by chromosomal testing in Japan (*22*) and one was similarly diagnosed outside Japan (*23*). Several WS cases have been diagnosed as pseudo-Klinefelter syndrome because of several clinical similarities such as slender extremities with stocky trunk, gynecomastia, and atrophic testis (*24,25*). However, chromosomal analysis of those cases indicated that they were normal males (*26*). The frequency of Klinefelter syndrome was about 1 in 700 live-born males in the general population, and the frequency among WS patients was about the same (*27*). In addition, one WS case of a patient with an extra chromosome was reported by Tanihara *et al*. in Japan (*28*).

Table 1. Mutation causes mutations?

Klinefelter syndrome	27M; 46/XY, 47/XXY: Funayama 1976 24M; 48/XXYY: Ferramosca 1972
Extrachromosome	43 F; 46/XX, 49/XX; Tanihara 1986
Retinitis pigmentosa	58M; Tajima 1993 39M; Valero 1960 38M; Kleeberg 1949
Wilson disease	55M; Sakai 1990
Xeroderma pigmentosum	23F; Takahashi 1955 29F; Takahashi 1955 29F; Takahashi 1955
Porokeratosis Mibelli	49M; Machino 1984

In 1 Japanese case and 2 European cases of WS, patients had retinitis pigmentosa (*29-31*). Retinitis pigmentosa has several types of transmission and an overall frequency of about 1 in 3,700 (*32*). Thus, an incidence of 2:350-1:1070 among WS patients was probably higher than in the general population.

In 1 Japanese case of WS, the patient had Wilson disease (*33*). The frequency of Wilson disease was about 1 in 30,000-100,000 livebirths worldwide (*32*). Of particular interest, 3 female siblings with WS may have the variant form of xeroderma pigmentosum (X-P) (*34,35*). The frequency of all types of X-P in Japan was about 1 in 40,000, which was higher than in the rest of the world (*27*). However, both the cases of WS and of X-P were clinically diagnosed 50 years ago.

WS has been classified as a genetic instability syndrome, which includes Bloom syndrome (*36,37*), Rothmund-Thomson syndrome (*38*), Cockayne syndrome (*37*), ataxia telangiectasia (*40,41*), X-P (*39*), Fanconi anemia (Alter BP, NCI personal communication), and progeria (*20*). However, no association of additional chromosomal abnormalities or genetic diseases with genetic instability syndromes was noted except for WS and Bloom syndrome. Machino reported a case of WS with porokeratosis Mibelli, and Takemiya described a case of Bloom syndrome with porokeratosis Mibelli (*42,43*). The frequency of porokeratosis Mibelli in the general population is not known.

In all of the cases analyzed, WS and additional genetic diseases such as retinitis pigmentosa, Wilson disease, and X-P were clinically determined, which merely suggests that WS has genetic instability when encountered clinically.

Finally, some of the aging-associated phenotypes seen may relate directly to WRN dysfunction. Aging is believed to induce genetic instability leading to cancer (*44,45*), and thus the complete loss of WRN function may epigenetically and genetically impact

other genes, promoters, or proteins related to aging-associated pathophysiology. It may also impact several disease-causing genes *via* acquired *in vivo* mosaicism or acquired *in vivo* mutation, as is reported in Rothmund-Thomson syndrome (*38*).

Medical researchers are encouraged to report cases of other genetic diseases or chromosomal abnormalities accompanying WS, as doing so may help to identify which diseases are associated with WS.

References

1. Comfort A. Werner's syndrome. Lancet 1961; ii:1152.
2. Fraccaro M, Bott MG, Calvert HT. Chromosomes in Werner's syndrome. Lancet 1962; i:536.
3. Learner N, Day HJ, Weiss L, Di George A. Chromosomes in Werrner's syndrome. Lancet 1962; i:537.
4. Motsulsky AG, Schultz A, Priest J. Werner's syndrome: chromosomes, genes, and the ageing process. Lancet 1962; i:160-161.
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. 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.
7. Goto M. Clinical characteristics of Werner syndrome and other premature aging syndromes: Pattern of aging in progeroid syndromes. In: Gann Monograph on Cancer Research No.49. From premature gray hair to helicase-Werner syndrome:Implications for aging and cancer (Goto M, Miller RW, ed.). Karger, Tokyo, 2001; pp. 27-39.
8. van Brabant AJ, Stan R, Ellis NA. DNA helicases, genomic instability, and human genetic disease. Annu Rev Genomics Hum Genet 2000; 1:409-459.
9. Fukuchi K, Martin GM, Monnat RJ. Mutator phenotype of Werner syndrome is characterized by extensive deletions. Proc Natl Acad Sci USA 1989; 86:5893-5897.
10. Kyoizumi S, Kusunoki Y, Seyama T, Hatamochi A, Goto M. *In vivo* somatic mutations in Werner's syndrome. Hum Genet 1998; 103:405-410.
11. Scappaticci S, Cerimele D, Fraccaro M. Clonal structural chromosomal rearrangements in primary fibroblast cultures and in lymphocytes of patients with Werner's syndrome. Hum Genet 1982; 62:16-24.
12. Hoehn H, Bryant EM, Au K, Norwood TH, Boman H, Martin GM. Variegated translocation mosaicism in human skin fibroblast cultures. Cytogenet Cell Genet 1975; 15:282-298.
13. Goto M. Hierarchical deterioration of body systems in Werner's syndrome: Implications for normal ageing. Mech Age Dev 1997; 98:239-254.
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, Miller R W, Ishikawa Y, Sugano H. Excess of rare cancers in Werner syndrome (Adult progeria). Cancer Epidemiol Biomarkers Prev 1996; 5:239-246.
16. Werner O. On cataract in conjunction with scleroderma. Doctoral dissertation. Kiel University. Schmidt and Klaunig, Kiel, 1904.
17. Goto M, Matsuura M. Secular trends towards delayed onsets of pathologies and prolonged longevities in Japanese patients with Werner syndrome. BioScience Trends 2008; 2:81-87.
18. Satoh M, Imai M, Sugimoto M, Goto M, Furuichi Y. Prevalence of Werner's syndrome heterozygotes in Japan. Lancet 1999; 353:1766.
19. 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: Gann Monograph on Cancer Research No.49. From premature gray hair to helicase-Werner syndrome:Implications for aging and cancer (Goto M, Miller RW, eds.). Karger, Tokyo, 2001; pp. 19-25.
20. Ellis NA. Mutation-causing mutations. Nature 1996; 381:110-111.
21. Ishida R. A case of cataract associated with scleroderma. Jap J Ophthalmol 1917; 21:1025-1032. (in Japanese)
22. Funayama S, Tsukashima T, Nakazawa Y. A case of Werner syndrome associated with Klinefelter syndrome. Diabetes 1976; 19:569-574. 46XY/47XXY mosaicism
23. Ferramosca B, Masiello O. An unusual case of corporeal dysmorphism with XXYY diploidy. Folia Endocrinol (Roma) 1972; 25:161-173.
24. Tritsch H, Lischka G. Werner syndrome combined with pseudo-Klinefelter syndrome. Hautarzt 1968; 12:547-551.
25. Lischka G, Tritsch H. Pseudo-Klinefelter syndrome with Werner syndrome. Hautarzt 1971; 3:122-125.
26. Aulepp H. Werner syndrome with pseudo-Klinefelter syndrome. Z Hautkr 1977; 52:362-364.
27. Scriver CR, Beaudet AL, Sly WS, Valle D. The metabolic basis of inherited disease I 6 edition. 1989.
28. Tanihara H, Chihara E. Complications and keratopathy following cataract surgery in a case with Werner's syndrome. Jap J Clin Ophthalmol 1986; 40:409-411.
29. Tajima H, Tuji M, Ebara T, Shikama Y, Adachi M. A case of Werner's syndrome with diabetes mellitus and hyperlipidemia. J Showa Med University 1993; 53:401-405.
30. Kleeberg J. A case of Werner's syndrome. Acta Med Orient (Tel-Aviv) 1949; 8-9:145-146.
31. Valero A, Gellei B. Retinitis pigmentosa, hypertension and uremia in Werner's syndrome. Report of a case with necropsy findings. Brit Med L 1960; 2:351-354.
32. McKusick VA. Mendelian inheritance in man. 8th edition. The Johns Hopkins University Press, 1988.
33. Sakai N, Honzyo K, Ono Y, Uehara S, Itagaki Y, Izumiyama S, Hirayama N, Ishikura M. A case of Werner syndrome associated with clinically diagnosed Wilson disease. Hepatology 1990; 31:95.
34. Takahashi H. Cataract associated with abnormal statute and skin changes. Proc Clin Ophthalmol 1955; 49:100-102.
35. Yasuoka T. Cataract associated with multiple endocrine dysfunction. Proc Clin Ophthalmol 1956; 50:551.
36. German J, Takebe H. Bloom's syndrome. XIV. The disoeder in Japan. Clin Genet 1989; 35:93-110.
37. German J, Sanz MM, Ciocci S, Ye TZ, Ellis NA. Syndrome-causing mutations of the BLM gene inpersons in the Bloom's syndrome registry. Hum Mutat 2007; 28:743-753.
38. Lindor NM, Devries EMG, Michels VV, Schad CR, Jalal SM, Donovan KM, Smithson WA, Kvols LK, Thibodeau SN, Dewald GW. Rothmund-Thomson syndrome in siblings: evidence for acquired *in vivo* mosaicism. Clin

Genet 1996; 49:124-129.
39. Kleijer WJ, Laugel V, Berneburg M, Nardo T, Fawcett H, Gratchev A, Jaspers NGJ, Sarasin A, Stefanini M, Lehmann AR. Incidence of DNA repair deficiency disorders in western Europe: Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. DNA Repair 2008; 7:744-750.
40. Meyn MS. High spontaneous intrachromosomal recombination rates in ataxia-telangiectasia. Science 1993; 260:1327-1330.
41. Shiloh Y. ATM and related protein kinases: Safegurding genome integrity. Nature Rev Cancer 2003; 3:155-168.
42. Machino H, Miki Y, Teramoto T, Shiraishi S, Sasaki MS. Cytogenetic studies in a patient with porokeratosis of Mibelli, multiple cancers and a forme fruste of Werner's syndrome. Brit J Dermatol 1984; 111:579-586.
43. Takemiya M, Shiraishi S, Teramoto T, Miki Y. Bloom's syndrome with porokeratosis of Mibelli and multiple cancers of the skin, lung and colon. Clin Genet 1987; 31:35-44.
44. Hirsch-Kauffmann M, Schweiger M. Aging and chromosomal instability. Rev Physiol Biochem Pharmacol 1999; 139:141-174.
45. Crabbe L, Jauch A, Naeger CM, Holtgreve-Grez H, Karlseder J. Telomere dysfunction as a cause of genomic instability in Werner syndrome. Proc Natl Acad Sci USA 2007; 104:2205-2210.

(*Received July 17, 2008; Revised July 28, 2008; Accepted July 29, 2008*)