Geriatrics & Gerontology International

DOI: 10.1111/ggi.14752

ORIGINAL ARTICLE

BIOLOGY

Sex differences in symptom presentation and their impact on diagnostic accuracy in Werner syndrome

Hiyori Kaneko,[1] Yoshiro Maezawa,[1] Ayano Tsukagoshi-Yamaguchi,[1] Masaya Koshizaka,[1] Aki Takada-Watanabe,[1] Rito Nakamura,[1] Shinichiro Funayama,[1] Kazuto Aono,[1] Naoya Teramoto,[1] Daisuke Sawada,[2] Yukari Maeda,[1] Takuya Minamizuka,[1] Aiko Hayashi,[1] Kana Ide,[1] Shintaro Ide,[1] Mayumi Shoji,[1] Takumi Kitamoto,[1] Minoru Takemoto,[3] Hisaya Kato[1] and Koutaro Yokote[1]

[1]Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, Chiba, Japan
[2]Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan
[3]Department of Diabetes, Metabolism and Endocrinology, International University of Health and Welfare, Chiba, Japan

Correspondence
Yoshiro Maezawa MD PhD, Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
Email: yoshiromaezawa@chiba-u.jp

Received: 1 July 2023
Revised: 27 October 2023
Accepted: 9 November 2023

Aim: Whether sex differences exist in hereditary progeroid syndromes remains unclear. In this study, we investigated sex differences in patients with Werner syndrome (WS), a model of human aging, using patient data at the time of diagnosis.

Methods: The presence of six cardinal signs in the diagnostic criteria was retrospectively evaluated.

Results: We found that the percentage of patients with all cardinal signs was higher in males than in females (54.2% vs. 21.2%). By the age of 40 years, 57.1% of male patients with WS presented with all the cardinal signs, whereas none of the female patients developed all of them. In particular, the frequency of having a high-pitched, hoarse voice, a characteristic of WS, was lower in female patients. The positive and negative predictive values for clinical diagnosis were 100% for males and females, indicating the helpfulness of diagnostic criteria regardless of sex. More female patients than male (86.7% vs. 64%) required genetic testing for their diagnosis because their clinical symptoms were insufficient, suggesting the importance of genetic testing for females even if they do not show typical symptoms of WS. Finally, the frequency of abnormal voice was lower in patients with WS harboring the c.3139-1G > C homozygous mutation.

Conclusion: These results indicate, for the first time, that there are sex differences in the phenotypes of hereditary progeroid syndromes. The analysis of this mechanism in this human model of aging may lead to the elucidation of sex differences in the various symptoms of normal human aging. **Geriatr Gerontol Int 2024; 24: 161–167**.

Keywords: abnormal voice, clinical diagnosis, diagnostic criteria, sex differences, Werner syndrome.

Introduction

As the world population ages, the interest in age-related diseases is growing. In recent years, it has become evident that age-related diseases such as atherosclerotic, metabolic, and neurodegenerative diseases exhibit sex differences.[1,2] Sex differences in life expectancy and healthy life expectancy have been recognized worldwide.[3]

Werner syndrome (WS) is a typical progeroid syndrome in which various signs of aging appear after puberty. It has attracted attention as a model of human aging. It is inherited as an autosomal recessive trait, and various aging phenomena, such as gray hair, bilateral cataracts, diabetes, arteriosclerosis, and skin ulcers, develop at an early age. The number of patients with WS in Japan is estimated to be 700–2000, and 60% of the world's patients have been reported in Japan. The syndrome is caused by a mutation in the *WRN* gene, which encodes a RecQ helicase on chromosome 8. The pathogenesis of this syndrome involves reduced DNA damage repair, telomere shortening, chronic inflammation, mitochondrial dysfunction, and epigenetic changes.[4–6] However, it remains unclear whether sex differences are observed in WS.

The main "cardinal" signs of WS are progeroid changes in the hair; bilateral cataracts; skin changes; intractable skin ulcers; calcification of the Achilles tendon; a birdlike face; and a high-pitched, hoarse voice. According to the 2012 Japanese WS Diagnostic Criteria, the syndrome can be diagnosed on the basis of clinical symptoms alone when all six "cardinal" signs are present. When some of the six signs are not present, a genetic diagnosis is required (Table 1).[7] However, whether sex differences exist in the frequency and timing of these aging symptoms remains unclear. Moreover, the usefulness of these diagnostic criteria and whether they are useful for both male and female patients have not been verified. Recently, the recognition of WS has increased, and the life expectancy of patients has increased from 40 to 59 years.[8,9] Accordingly,

Table 1 Diagnostic criteria for Werner syndrome 2012

I. Cardinal signs and symptoms (onset occurring after the age of 10 years until 40 years)	
1. Progeroid changes of hair	Gray hair, baldness, etc.
2. Cataract	Bilateral
3. Changes of skin, intractable skin ulcers	Atrophic skin, tight skin, clavus, callus
4. Soft-tissue calcification	Achilles tendon, etc.
5. Birdlike face	
6. Abnormal voice	High-pitched, squeaky, hoarse voice
II. Other signs and symptoms	
1. Abnormal glucose and/or lipid metabolism	
2. Deformation and abnormality of the bone	Osteoporosis, etc.
3. Malignant tumors	Nonepithelial tumors
4. Parental consanguinity	
5. Premature atherosclerosis	Angina pectoris, myocardial infarction
6. Hypogonadism	
7. Short stature and low body weight	
III. Genetic testing	

Addendum: Mental retardation is seldom observed in patients with Werner syndrome, and their cognitive function is often appropriate for their age. Confirmed: Presence of all cardinal signs or confirmation of biallelic *WRN* mutations and at least three cardinal signs. Suspected: Presence of both I-1 (progeroid changes in hair) and I-2 (cataracts) in conjunction with at least two signs or symptoms other than I or II.

it is assumed that the age and symptoms of patients are more diverse than when the diagnostic criteria were established.

Therefore, we investigated whether there are sex differences in the aging progression of WS by examining the positivity ratio and timing of the appearance of the cardinal signs. We also investigated the validity of the current diagnostic criteria in both male and female patients.

Methods

We evaluated the clinical symptoms described in the referral letters of patients whose primary physicians suspected WS and requested genetic diagnosis at Chiba University Hospital. A total of 170 patients whose requests were received from 2009 to January 2022 were included, and a genetic diagnosis was performed for all patients. Clinical manifestations, especially the cardinal signs of WS and age, were evaluated on the basis of the time when the genetic diagnosis was performed. Clinical symptoms were independently reviewed by two physicians with experience in treating patients with WS. We excluded 78 patients for whom the clinical classification could not be determined because of insufficient information about symptoms in the referral letters. Ultimately, we analyzed the symptoms and genetic results of 92 patients.

Analysis was performed using Pearson's chi-squared test, and a P-value of <0.05 was considered statistically significant. This study was approved by the Ethics Committee of Chiba University Hospital (approval no. 1145 on October 21, 2021) and conducted in accordance with the Declaration of Helsinki.

Results

Of the 170 patients with suspected WS who were referred to our hospital between 2009 and 2022, 92 with detailed clinical data available were included. Of the 92 patients in the study, 48 (52.2%) were men and 44 (47.8%) were women. They had a median age at genetic diagnosis of 44.5 years (interquartile range [IQR], 40–51 years), with a median age at genetic diagnosis for men of 43.5 years (IQR, 39.8–50 years) and 45.5 years (IQR, 40–52.3 years) for women. The patients were divided into three categories according to the clinical components of the diagnostic criteria. The "definite" category included patients with all the cardinal signs. The "probable" category included those with three cardinal signs or those who had hair changes and cataracts plus at least two more signs. The "excluded" category consisted of patients who did not meet the criteria for the first two categories. All the patients with positive genetic test results were diagnosed with WS.

First, we examined the categories of clinical diagnoses according to the sex of the patients who had a positive genetic test. The proportion of clinically definite was 19 of 35 (54.2%) men and 7 of 33 (21.2%) women, indicating that male patients with WS were significantly more likely to have all the cardinal signs than female patients ($\chi^2[1] = 7.867$; $P = 0.0050$; $\varphi = 0.340$; Fig. 1a). When comparing patients with WS who were aged < 40 years, 4 of 7 (57.1%) men were definite, whereas none of the six women were definite ($\chi^2[1] = 4.952$; $P = 0.026$; $\varphi = 0.617$; Fig. 1b). Furthermore, we evaluated the age distribution of definite and probable male and female patients (Fig. 1c). The histogram shows that male patients with WS tend to develop all the cardinal signs in their 40th year, whereas many female patients lacked some of the cardinal signs and were categorized as probable until their 50s. These results suggest that men with WS are more likely to develop all the cardinal signs earlier than women.

We examined the positivity ratio (number of patients with clinical symptoms/number of patients who underwent the examination) and inspection ratio (number of patients who underwent the examination/total number of patients) for each cardinal sign in patients with WS (Table 2). The positivity ratio for abnormal voice was as low as 73.2% among the patients with definite WS and only 50% for clinically probable patients. In addition, the positivity ratio for abnormal voice was lower in female patients (15/26 [57.7%]) than in male patients (26/30 [86.7%]; $\chi^2[1] = 5.963$; $P = 0.015$; $\varphi = 0.326$). The positivity ratio of abnormal voice in male patients in their 20s, 30s, and 40s was 100%, 100%, and 92.3%, respectively. In comparison, the positivity ratio for female patients in their 20s, 30s, and 40s was 0%, 50%, and 69.2%, respectively ($\chi^2[1] = 4.690$; $P = 0.016$; $\varphi = 0.685$; Fig. 2a; Table S1.). Comparison of male and female patients aged <40 years showed that seven of seven male patients and only one of three female patients were positive for abnormal voice (Fig. 2b). Thus, female patients with WS showed delayed development of abnormal voice.

To validate the accuracy of diagnostic criteria for both sexes, we compared the categories of clinical diagnoses, with a positive genetic test as a confirmed diagnosis (Table 3). Of the 26 patients categorized as definite, all 26 were confirmed to have WS on genetic testing. For the 11 excluded patients, the genetic test was negative in all, demonstrating remarkable concordance between the clinical category and genetic test. Two of the excluded patients had a non-Werner genetic progeroid disease. One patient had atypical WS with generalized lipodystrophy and a de novo heterozygous *LMNA* p.T10I mutation.[10] One patient harbored a *POLD1* mutation. Both patients were diagnosed as non-WS because of the absence of cataracts.

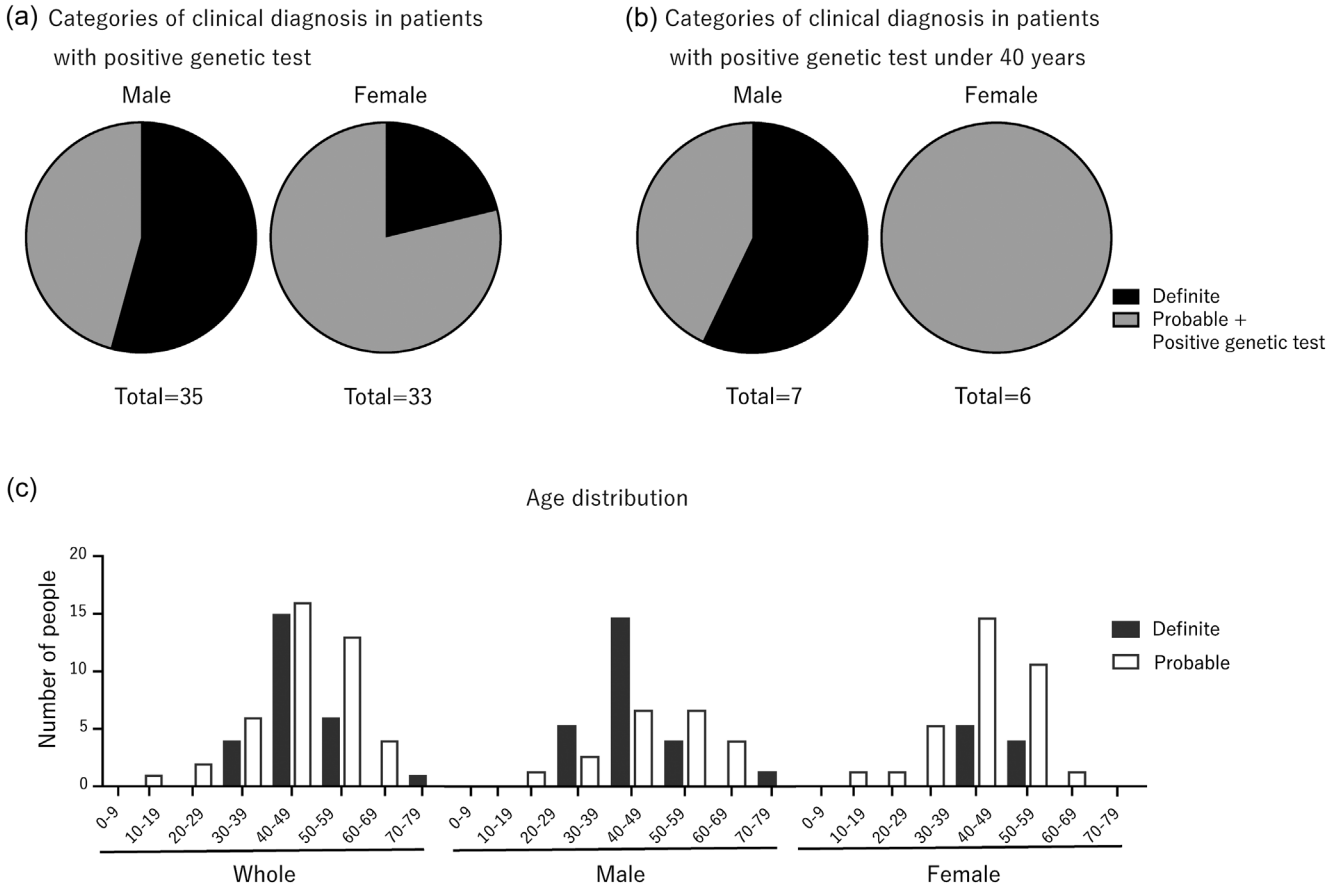

Figure 1 (a) Categories of clinical diagnosis in patients with positive genetic test. (b) Categories of clinical diagnosis in patients with positive genetic test <40 years of age. (c) Age distribution of definite and probable patients by sex in genetically positive patients.

Table 2 Positivity ratios and inspection ratios for each cardinal sign of genetic test-positive patients

		Progeroid changes of hair	Cataracts	Changes of skin, intractable skin ulcers	Achilles tendon calcification	Birdlike face	Abnormal voice
Overall	Positivity ratio	98.4 (60/61)	98.5 (64/65)	94 (63/67)	96.4 (53/55)	93.1 (54/58)	73.2 (42/56)
	Inspection ratio	89.7 (61/68)	95.6 (65/68)	98.5 (67/68)	80.9 (55/68)	85.3 (58/68)	82.4 (56/68)
Probable	Positivity ratio	97.1 (34/35)	97.4 (38/39)	88.1 (37/42)	78.1 (25/32)	87.5 (28/32)	50 (15/30)
	Inspection ratio	83.7 (36/43)	92.9 (39/42)	97.6 (41/42)	76.2 (32/42)	76.2 (32/42)	71.4 (30/42)
Male	Positivity ratio	96.9 (31/32)	100 (34/34)	94.1 (32/34)	96.6 (28/29)	96.7 (29/30)	86.7 (26/30)
	Inspection ratio	91.4 (32/35)	97.1 (34/35)	97.1 (34/35)	82.9 (29/35)	85.7 (30/35)	85.7 (30/35)
Female	Positivity ratio	100 (29/29)	96.8 (30/31)	93.9 (31/33)	96.2 (25/26)	89.3 (25/28)	57.7 (15/26)
	Inspection ratio	87.9 (29/33)	93.9 (31/33)	100 (33/33)	78.8 (26/33)	84.8 (28/33)	78.8 (26/33)

Positivity ratio % (number of patients with clinical symptoms/number of patients who underwent the examination).

Inspection ratio % (number of patients who underwent the examination/total number of patients).

Of the 55 clinically probable patients, 42 (76.4%) were positive on genetic testing (Table 3A). When examined by sex, the positive and negative predictive values for "definite" and "excluded" by clinical symptoms were 100.0% for both men and women (Table 3B,C). Among the probable patients, the positivity ratio of the genetic test was 64.0% in men (16/25 patients) and 86.7% in women (26/30 patients) (Table 3B,C) ($\chi^2[1] = 3.882$; $P = 0.049$; $\varphi = 0.071$). Given that the current diagnostic criteria were revised in 2012, we selected 81 patients who were diagnosed after establishment of the current diagnosis and examined the validity of the diagnostic criteria (Table S2A–C). We found positive and negative predictive values were 100%, even when restricted from 2012 to 2022.

An abnormal voice and a "birdlike face" can be a subjective sign. Therefore, we examined the accuracy of the diagnoses when either an abnormal voice (Table S3A) or a birdlike face (Table S3B) was deleted from the cardinal signs and when both were deleted (Table S3C). First, when the abnormal voice was

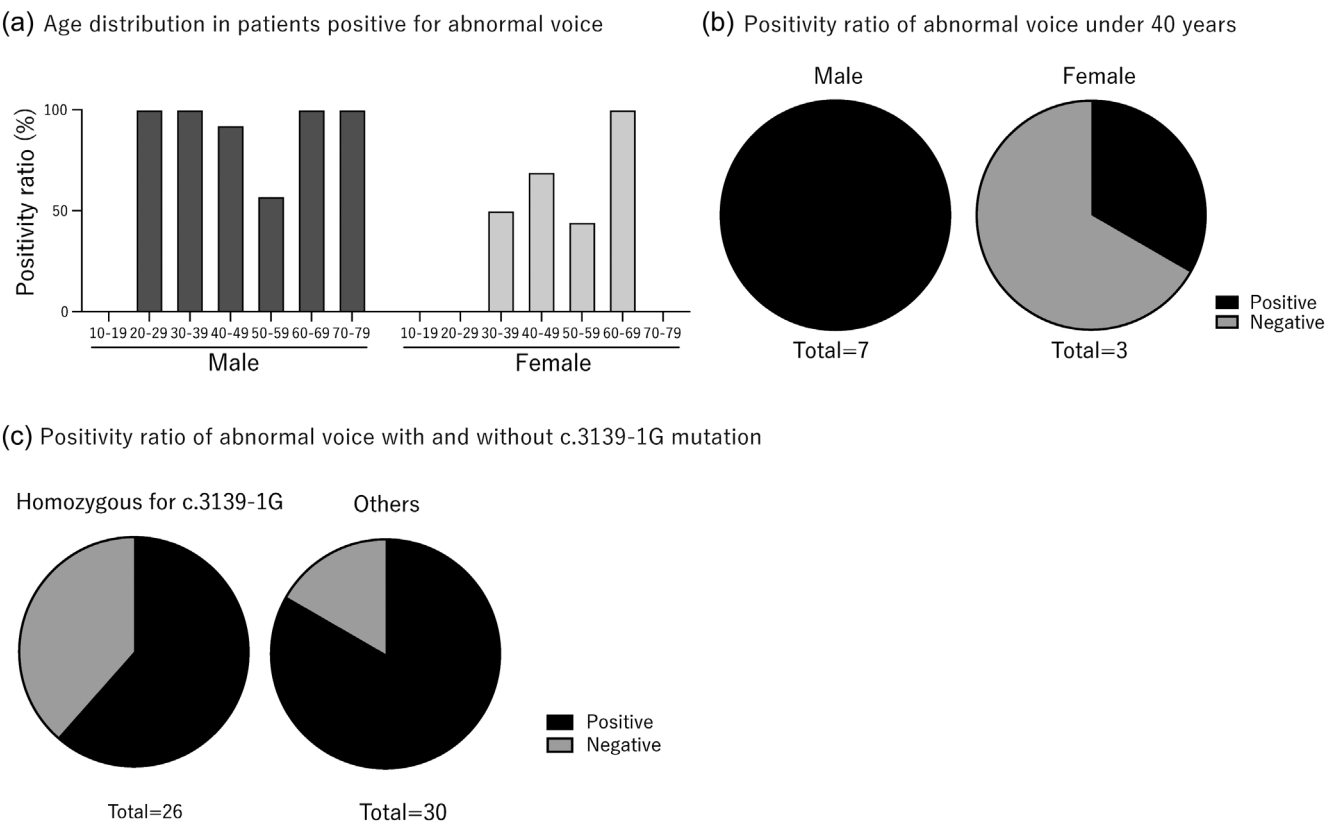

Figure 2 (a) Age distribution in patients positive for abnormal voice. (b) Positivity ratio of abnormal voice <40 years. (c) Positivity ratio of abnormal voice with and without the c.3139-1G mutation homozygous for c.3139-1G.

deleted from the cardinal signs, the positive predictive value was 100%; however, the negative predictive value was reduced to 91.7%. Similarly, when the birdlike face was deleted, the positive predictive value was 100%, but the negative predictive value was reduced to 85.7%. Finally, when both were deleted, the positive predictive value was 100%, and the negative predictive value was reduced to 78.9% (Table S3A–C). These results indicate the necessity of including the birdlike face and abnormal voice for the accuracy of the criteria.

Finally, we examined other factors that affect the frequency of abnormal voices and found that the site of the gene mutation was associated with its presence. More than 80 *WRN* mutations have been reported, of which c.3139-1G > C is the most frequent in Japanese patients with WS.[11] In this study, of the 68 patients with a positive genetic test, clinical data on abnormal voice were available for 56. We analyzed the association between homozygosity for the c.3139-1G > C mutation and the presence of an abnormal voice. The results showed that patients homozygous for c.3139-1G > C exhibited an abnormal voice less frequently ($\chi^2[1] = 5.482$; $P = 0.019$; $\chi = 0.313$) (Fig. 2c), and the proportion of homozygosity did not differ between sexes ($\chi^2[1] = 0.249$; $P = 0.618$; $\chi = 0.067$). The actual number of patients is shown in Table S4. Therefore, the site of the genetic mutation influences the occurrence of abnormal voices.

Discussion

In this study, we demonstrated that the main symptoms of WS appear earlier and more frequently in males. In addition, we showed that the characteristic high-pitched, hoarse voice is less frequent in women, especially in patients with the c.3139-1G > C homozygous founder mutation. This is the first report of sex differences in hereditary progeroid syndromes caused by a single gene mutation and provides a new perspective on the similarities between general aging and genetic progeroid syndromes. We also revealed, for the first time, that the phenotype of WS differs depending on genetic mutations. This provides new insight into the partial functionality of the WRN mutant protein, contrary to the conventional view that the symptoms of WS are the same regardless of the genetic mutation.

As average life expectancy increases, interest in sex differences in longevity and age-related diseases, such as metabolic syndrome and atherosclerosis, is increasing.[12,13] However, Goto *et al.*, in their 1966 to 2004 study of 1019 Japanese patients with WS, reported an average life expectancy of 51.9 years for women compared to 53.6 years for men, with no sex difference.[14] However, in that study, most patients were diagnosed only clinically, and a genetic confirmation was not obtained.[14] There have been no reports concerning sex differences in other progeroid syndromes, including Hutchinson–Gilford progeria and Cockayne syndromes.[15,16] This study shows, for the first time, the sex differences in hereditary progeroid syndromes using patient data with a genetically confirmed diagnosis.

In general, women live longer than men. Mitochondrial dysfunction and shortened telomeres have been reported as hallmarks of aging,[17] and women have longer telomeres and greater amounts of mitochondrial DNA.[18] In addition, postmenopausal women who receive estrogen supplementation have lower epigenetic age and reduced cardiac disease and total mortality.[19] Furthermore,

Table 3 Definite cutoff.

A. Results of genetic testing for each clinical diagnosis group				
			Positive	Negative
Clinical symptoms		Definite	26	0
		Probable	42	13
		Excluded	0	11
			Positive predictive value	Negative predictive value
Definite cutoff	(%)		100	36.4
	Number of patients		26/26	13 + 11/42 + 13 + 11
Probable cutoff	(%)		84	100
	Number of patients		26 + 42/24 + 42 + 13	11/11
B. Results of genetic testing for each male clinical diagnosis group				
			Positive	Negative
Clinical symptoms		Definite	19	0
		Probable	16	9
		Excluded	0	4
			Positive predictive value (%)	Negative predictive value (%)
Definite cutoff	(%)		100	44.8
	Number of patients		19/19	9 + 4/16 + 9 + 4
Probable cutoff	(%)		79.5	100
	Number of patients		19 + 16/19 + 16 + 9	4/4
C. Results of genetic testing for each female clinical diagnosis group				
			Positive	Negative
Clinical symptoms		Definite	7	0
		Probable	26	4
		Excluded	0	7
			Positive predictive value (%)	Negative predictive value (%)
Definite cutoff	(%)		100	29.7
	Number of patients		7/7	4 + 7/26 + 4 + 7
Probable cutoff	(%)		89.2	100
	Number of patients		7 + 26/7 + 26 + 4	7/7

When the cutoff is set between "Definite" and "Probable," positive predictive value includes Definite, and negative predictive value includes Probable and "Excluded." Probable cutoff: When the cutoff is set between Probable and Excluded, positive predictive value includes Definite and Probable, and negative predictive value includes Excluded.

telomere length and epigenetic age correlate with cardiovascular disease and metabolic disease.[20,21] Thus, in women, there are estrogen-dependent and -independent, cellular, and individual suppression mechanisms on aging. Telomere shortening, mitochondrial dysfunction, and increased epigenomic age are also critical in WS. The presence of premature menopause in patients with WS suggests that the protective effect of estrogen is lost in the 30s. On the other hand, delayed symptoms in the current study suggest a protective effect on aging that persists even after menopause.

Estrogen potentially has a protective effect on hair loss, skin atrophy, and cataracts.[22,23] Estrogen levels affect subcutaneous fat deposition in women, and visceral fat accumulation and insulin resistance occur more frequently in men.[24,25] The frequency of cardiovascular disease is low in women before menopause.[26,27] Age-related decrease in estimated glomerular filtration rate is greater in men.[28–30] Refractory skin ulcers are often observed in patients with WS, and a microarray study of skin wound healing in older men reported that 76% of the identified aging-related genes were estrogen regulated.[31] At the molecular level, age-related reductions in double-strand break repair and increased telomere uncapping, which are also characteristic of WS, are more severe in postmenopausal women than in men.[32,33] These findings suggest that estrogen may contribute to delayed aging symptoms of WS in women. However, the effect of estrogen on birdlike face and abnormal voice may not be likely because it is specific to WS.

Interestingly, WRN expression is upregulated following estrogen administration,[34] suggesting that *WRN* upregulation by estrogen contributes to double-strand break repair and telomere maintenance in women. However, Imura *et al.* reported that follicle-stimulating hormone and luteinizing hormone are elevated in patients with WS in their 30s and 40s, suggesting primary hypogonadism at an early age. Serum testosterone levels were lower than in age-matched controls, and testicular biopsies showed pronounced atrophy. Therefore, it may be difficult to attribute the delay of WS manifestations in women only to sex hormones.[35] Various factors, including chromosome structure, mitochondrial genetic format, and mitochondrial DNA content, also influence sex differences in aging other than estrogen.[36,37] Taken together, these results suggest that estrogen-independent mechanisms may also be involved.

A high-pitched, hoarse voice is a characteristic clinical finding of WS.[38] In normal aging, the voice tends to become hoarse as vocal cord atrophy progresses.[39] While men acquire a high-pitched voice due to the hardening of the vocal muscles by aging, women acquire a low-pitched voice due to edema and thickening of the vocal folds caused by a decrease in the female hormones.[40] Therefore, the less frequent high-pitched voices in women with WS are consistent with age-related changes. Also, our data (Fig. 2) suggest that abnormal voice increases with age, and it occurs the latest among the various manifestations of WS. Among the cardinal signs, high-pitched, hoarse voices and birdlike faces are specific to WS rather than normal aging, yet they still appear according to age.

In this study, the positive and negative predictive values for the definite and excluded categories were 100% for both sexes, suggesting that these diagnostic criteria are helpful regardless of sex. On the other hand, the positivity ratio of genetic diagnosis in the probable category was 76.4%, suggesting the importance of genetic diagnosis when clinical symptoms are insufficient. In addition, more female patients required genetic testing, indicating the importance of genetic testing in females even if they do not show typical symptoms. These criteria could not detect atypical WS or *POLD1* mutations, suggesting that an additional strategy is required to detect rare non-Werner progeroid patients.

This study had some limitations. First, this was a retrospective cohort study; therefore, a prospective study is needed to confirm these results. Additionally, the number of participating patients was small because WS is a rare disease. Moreover, the present study focused only on the occurrence of the six cardinal signs because it is based on the information in the referral letter for request of genetic diagnosis; we could not describe the detailed clinical data such as comorbidities, body composition, or onset and progression of diabetes or atherosclerosis.

In conclusion, this study, for the first time, showed that sex differences exist in WS symptoms. In addition, sex differences were found for occurrence of abnormal voices in the cardinal signs and were influenced by genotype. Further detailed studies are required to clarify whether the sex differences observed in this study are specific to WS or applicable to general aging.

Acknowledgements

The authors thank all the staff and patients who contributed to this study.

Funding information

This work was supported by KAKENHI JP22K20844 (H. Kaneko), JP22K15682 (H. Kato), JP21K19437 (K.Y.), JP23H00417 (K.Y.); AMED JP21jm0210096 (K.Y.), JP22ym0126066 (K.Y.), JP23ek0109622 (K.Y.); and MHLW of Japan JPMH21FC1016 (K.Y.).

Disclosure statement

All authors declare no conflicts of interest and nothing to disclose.

Author contributions

H. Kaneko, A.T.Y., H. Kato, M.K., and K.Y. managed the project. H. Kaneko, A.T.Y., H. Kato, M.S., Y. Maeda, M.K., and M.T. recruited the patients. H. Kaneko, H. Kato, M.K., A.T.W., R.N., S.F., K.A., and N.T. collected the data. H. Kaneko, H. Kato, M.K., D.S., T.M., A.H., K.I., S.I., and T.K. analyzed the data. H. Kato and Y. Maezawa directed this research. H. Kaneko, H. Kato, and Y. Maezawa wrote the manuscript. All authors read and discussed the manuscript.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1 Ostan R, Monti D, Gueresi P, Bussolotto M, Franceschi C, Baggio G. Gender, aging and longevity in humans: an update of an intriguing/neglected scenario paving the way to a gender-specific medicine. *Clin Sci (Lond)* 2016; **130**: 1711–1725. https://doi.org/10.1042/cs20160004.
2 Regitz-Zagrosek V. Sex and gender differences in health. *EMBO Rep* 2012; **13**: 596–603. https://doi.org/10.1038/embor.2012.87.
3 Ginter E, Simko V. Women live longer than men. *Bratisl Lek Listy* 2013; **114**: 45–49. https://doi.org/10.4149/bll_2013_011.
4 Kato H, Maezawa Y, Takayama N *et al.* Fibroblasts from different body parts exhibit distinct phenotypes in adult progeria Werner syndrome. *Aging (Albany NY)* 2021; **13**: 4946–4961. https://doi.org/10.18632/aging.202696.
5 Kato H, Maezawa Y, Ouchi Y *et al.* Generation of disease-specific and CRISPR/Cas9-mediated gene-corrected iPS cells from a patient with adult progeria Werner syndrome. *Stem Cell Res* 2021; **53**: 102360. https://doi.org/10.1016/j.scr.2021.102360.
6 Fang EF, Hou Y, Lautrup S *et al.* NAD(+) augmentation restores mitophagy and limits accelerated aging in Werner syndrome. *Nat Commun* 2019; **10**: 5284. https://doi.org/10.1038/s41467-019-13172-8.
7 Takemoto M, Mori S, Kuzuya M *et al.* Diagnostic criteria for Werner syndrome based on Japanese nationwide epidemiological survey. *Geriatr Gerontol Int* 2013; **13**: 475–481. https://doi.org/10.1111/j.1447-0594.2012.00913.x.
8 Koshizaka M, Maezawa Y, Maeda Y *et al.* Time gap between the onset and diagnosis in Werner syndrome: a nationwide survey and the 2020 registry in Japan. *Aging* 2020; **12**: 24940–24956. https://doi.org/10.18632/aging.202441.
9 Kato H, Koshizaka M, Kaneko H, Maezawa Y, Yokote K. Lifetime extension and the recent cause of death in Werner syndrome: a retrospective study from 2011 to 2020. *Orphanet J Rare Dis* 2022; **17**: 226. https://doi.org/10.1186/s13023-022-02383-w.
10 Fukaishi T, Minami I, Masuda S *et al.* A case of generalized lipodystrophy-associated progeroid syndrome treated by leptin replacement with short and long-term monitoring of the metabolic and endocrine profiles. *Endocr J* 2020; **67**: 211–218. https://doi.org/10.1507/endocrj.EJ19-0226.
11 Yokote K, Chanprasert S, Lee L *et al.* WRN mutation update: mutation Spectrum, patient registries, and translational prospects. *Hum Mutat* 2017; **38**: 7–15. https://doi.org/10.1002/humu.23128.
12 Rubin JB, Lagas JS, Broestl L *et al.* Sex differences in cancer mechanisms. *Biol Sex Differ* 2020; **11**: 17. https://doi.org/10.1186/s13293-020-00291-x.
13 Man JJ, Beckman JA, Jaffe IZ. Sex as a biological variable in atherosclerosis. *Circ Res* 2020; **126**: 1297–1319. https://doi.org/10.1161/circresaha.120.315930.
14 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.
15 Prakash A, Gordon LB, Kleinman ME *et al.* Cardiac abnormalities in patients with Hutchinson-Gilford progeria syndrome. *JAMA Cardiol* 2018; **3**: 326–334. https://doi.org/10.1001/jamacardio.2017.5235.
16 Natale V, Raquer H. Xeroderma pigmentosum-Cockayne syndrome complex. *Orphanet J Rare Dis* 2017; **12**: 65. https://doi.org/10.1186/s13023-017-0616-2.
17 Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013; **153**: 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039.

18 Factor-Litvak P, Susser E, Kezios K *et al*. Leukocyte telomere length in newborns: implications for the role of telomeres in human disease. *Pediatrics* 2016; **137**: e20153927. https://doi.org/10.1542/peds.2015-3927.
19 Mikkola TS, Tuomikoski P, Lyytinen H *et al*. Estradiol-based postmenopausal hormone therapy and risk of cardiovascular and all-cause mortality. *Menopause* 2015; **22**: 976–983. https://doi.org/10.1097/GME.0000000000000450.
20 Haycock PC, Heydon EE, Kaptoge S, Butterworth AS, Thompson A, Willeit P. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* 2014; **349**: g4227. https://doi.org/10.1136/bmj.g4227.
21 Muñoz-Lorente MA, Cano-Martin AC, Blasco MA. Mice with hyper-long telomeres show less metabolic aging and longer lifespans. *Nat Commun* 2019; **10**: 4723. https://doi.org/10.1038/s41467-019-12664-x.
22 Hales AM, Chamberlain CG, Murphy CR, McAvoy JW. Estrogen protects lenses against cataract induced by transforming growth factor-beta (TGFbeta). *J Exp Med* 1997; **185**: 273–280. https://doi.org/10.1084/jem.185.2.273.
23 McRobb LS, McGrath KCY, Tsatralis T *et al*. Estrogen receptor control of atherosclerotic calcification and smooth muscle cell osteogenic differentiation. *Arterioscler Thromb Vasc Biol* 2017; **37**: 1127–1137. https://doi.org/10.1161/ATVBAHA.117.309054.
24 Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med* 2009; **6**: 60–75. https://doi.org/10.1016/j.genm.2009.02.002.
25 Comitato R, Saba A, Turrini A, Arganini C, Virgili F. Sex hormones and macronutrient metabolism. *Crit Rev Food Sci Nutr* 2015; **55**: 227–241. https://doi.org/10.1080/10408398.2011.651177.
26 Iorga A, Cunningham CM, Moazeni S, Ruffenach G, Umar S, Eghbali M. The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biol Sex Differ* 2017; **8**: 33. https://doi.org/10.1186/s13293-017-0152-8.
27 Morselli E, Frank AP, Santos RS, Fátima LA, Palmer BF, Clegg DJ. Sex and gender: critical variables in pre-clinical and clinical medical research. *Cell Metab* 2016; **24**: 203–209. https://doi.org/10.1016/j.cmet.2016.07.017.
28 Melsom T, Norvik JV, Enoksen IT *et al*. Sex differences in age-related loss of kidney function. *J Am Soc Nephrol* 2022; **33**: 1891–1902. https://doi.org/10.1681/asn.2022030323.
29 Baylis C. Sexual dimorphism, the aging kidney, and involvement of nitric oxide deficiency. *Semin Nephrol* 2009; **29**: 569–578. https://doi.org/10.1016/j.semnephrol.2009.07.003.
30 Shuster BZ, Depireux DA, Mong JA, Hertzano R. Sex differences in hearing: probing the role of estrogen signaling. *J Acoust Soc Am* 2019; **145**: 3656–3663. https://doi.org/10.1121/1.5111870.
31 Hardman MJ, Ashcroft GS. Estrogen, not intrinsic aging, is the major regulator of delayed human wound healing in the elderly. *Genome Biol* 2008; **9**: R80. https://doi.org/10.1186/gb-2008-9-5-r80.
32 Rall-Scharpf M, Friedl TWP, Biechonski S, Denkinger M, Milyavsky M, Wiesmüller L. Sex-specific differences in DNA double-strand break repair of cycling human lymphocytes during aging. *Aging (Albany NY)* 2021; **13**: 21066–21089. https://doi.org/10.18632/aging.203519.
33 Walker AE, Morgan RG, Ives SJ *et al*. Age-related arterial telomere uncapping and senescence is greater in women compared with men. *Exp Gerontol* 2016; **73**: 65–71. https://doi.org/10.1016/j.exger.2015.11.009.
34 Lee SJ, Lee SH, Ha NC, Park BJ. Estrogen prevents senescence through induction of WRN, Werner syndrome protein. *Horm Res Paediatr* 2010; **74**: 33–40. https://doi.org/10.1159/000313366.
35 Imura H, Nakao Y, Kuzuya H, Okamoto M, Okamoto M, Yamada K. Clinical, endocrine and metabolic aspects of the Werner syndrome compared with those of normal aging. *Adv Exp Med Biol* 1985; **190**: 171–185. https://doi.org/10.1007/978-1-4684-7853-2_6.
36 Gentilini D, Castaldi D, Mari D *et al*. Age-dependent skewing of X chromosome inactivation appears delayed in centenarians' offspring. Is there a role for allelic imbalance in healthy aging and longevity? *Aging Cell* 2012; **11**: 277–283. https://doi.org/10.1111/j.1474-9726.2012.00790.x.
37 Hägg S, Jylhävä J, Wang Y, Czene K, Grassmann F. Deciphering the genetic and epidemiological landscape of mitochondrial DNA abundance. *Hum Genet* 2021; **140**: 849–861. https://doi.org/10.1007/s00439-020-02249-w.
38 Tsunoda K, Takanosawa M, Kurikawa Y, Nosaka K, Niimi S. Hoarse voice resulting from premature ageing in Werner's syndrome. *J Laryngol Otol* 2000; **114**: 61–63. https://doi.org/10.1258/0022215001903708.
39 Takano S, Kimura M, Nito T, Imagawa H, Sakakibara K, Tayama N. Clinical analysis of presbylarynx – vocal fold atrophy in elderly individuals. *Auris Nasus Larynx* 2010; **37**: 461–464. https://doi.org/10.1016/j.anl.2009.11.013.
40 Lenell C, Sandage MJ, Johnson AM. A tutorial of the effects of sex hormones on laryngeal senescence and neuromuscular response to exercise. *J Speech Lang Hear Res* 2019; **62**: 602–610. https://doi.org/10.1044/2018_jslhr-s-18-0179.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website:

Supplementary Table S1. Inspection and positivity ratios for abnormal voices in male patients with positive genetic test results.

How to cite this article: Kaneko H, Maezawa Y, Tsukagoshi-Yamaguchi A, et al. Sex differences in symptom presentation and their impact on diagnostic accuracy in Werner syndrome. Geriatr. Gerontol. Int. 2024;24:161–167. https://doi.org/10.1111/ggi.14752