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[CASE REPORT]

Characteristic Clinical Features of Werner Syndrome with a Novel Compound Heterozygous WRN Mutation c.1720+1G>A Plus c.3139-1G>C

Namiko Matsumoto[1,2], Yasuyuki Ohta[1], Kentaro Deguchi[2], Masayuki Kishida[3], Kota Sato[1], Jingwei Shang[1], Mami Takemoto[1], Nozomi Hishikawa[1], Toru Yamashita[1], Aki Watanabe[4], Koutaro Yokote[4], Minoru Takemoto[5], Junko Oshima[6] and Koji Abe[1]

Abstract:

Werner syndrome (WS) is an autosomal recessive progeroid disorder caused by mutations in the WRN gene (WRN). Most Japanese WS patients are born from a consanguineous marriage with homozygous WRN mutations. We herein report a rare WS patient born from non-consanguineous parents with compound heterozygous WRN mutations with a novel heterogeneous c.1720+1G>A substitution plus the most frequent heterogeneous c.3139-1G>C substitution among Japanese. Although the present case showed clinical characteristics common to previous Japanese WS patients, he had not developed any malignant tumors as of 43 years of age, suggesting that WS patients with this particular genetic mutation have a different phenotype than others.

Key words: werner syndrome, compound heterozygous, Japanese

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Introduction

Werner syndrome (WS) is an autosomal recessive progeroid disorder. The majority of WS patients are born from a consanguineous marriage with homozygous WRN gene (WRN) mutations and usually show similar clinical features, such as a senile appearance, subcutaneous calcification, painful ulcers, age-related disorders and malignant tumors (1). We herein report a rare WS patient with compound heterozygous WRN mutations, including a novel heterogeneous c.1720+1G>A substitution plus the most frequent heterogeneous c.3139-1G>C substitution among Japanese (2). The patient, who is now 43 years old, has not yet developed any malignant tumors.

Case Report

An eight-year-old Japanese boy noticed pain in his posterior neck and lumbar region, and the pain had spread throughout his body by the time he was 13. He subsequently developed bilateral cataracts at the age of 19 and gray hair and baldness by the time he turned 25. At 30 years of age, his pain became much more acute, especially in the bilateral Achilles tendons. At 38 years of age, he was admitted to our hospital for examinations. There was no family history of progeroid syndrome, age-related disorders or consanguineous marriage (Figure A).

He was 162 cm tall and weighed 50 kg. A physical examination revealed that he had a "bird-like face", senile appearance (Figure B), a high-pitched voice and hyperkeratosis in the bilateral elbows (Figure C, arrows), knees (Figure D,

[1]Department of Neurology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan, [2]Departments of Neurology, Okayama Citizen's Hospital, Japan, [3]General Internal Medicine, Okayama Citizen's Hospital, Japan, [4]Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan, [5]Department of Diabetes, Metabolism and Endocrinology, School of Medicine, International University of Health and Welfare, Japan and [6]Department of Pathology, University of Washington, USA
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Correspondence to Dr. Koji Abe, plrg69bp@cc.okayama-u.ac.jp

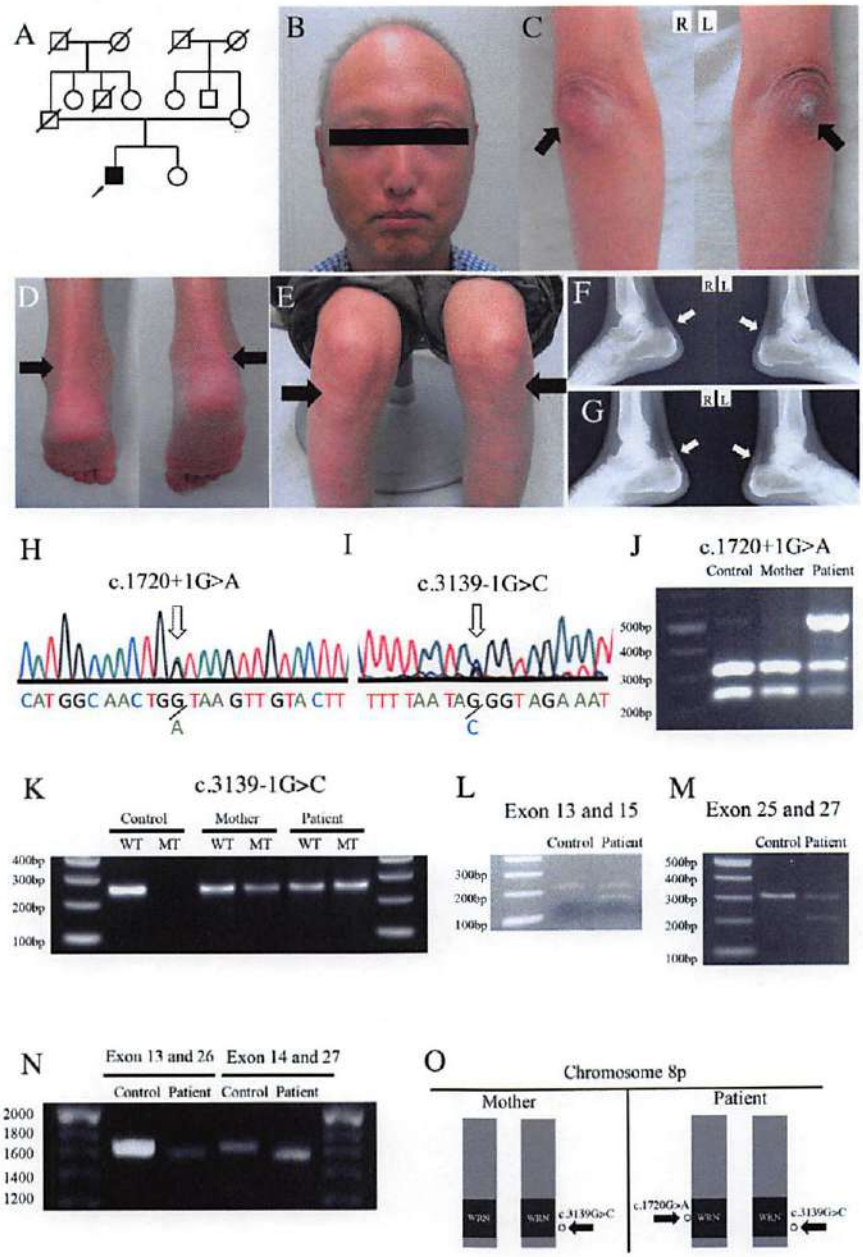

Figure. The patient's (A) family tree, (B) characteristic "bird-like face" and senile appearance, (C, arrows) cutaneous hyperkeratosis in bilateral elbows, (D, arrows) knees and (E, arrows) ankles. (F and G, arrows) X-ray showing the segmented calcification of the Achilles tendons at the diagnosis and five years later. The patient's (H and I, arrows) direct DNA sequence of the WRN gene revealed a c.1720+1G>A substitution in intron 14 and c.3139-1G>C substitution in intron 25 (2), which was (J) confirmed by PCR-RFLP and (K) AS-PCR for the patient and his mother. RT-PCR spanning (L) exons 13 to 15, (M) exons 25 to 27, (N) exons 13 to 26 and exons 14 to 27, confirming that (O) skips in exons 14 and 26 take place in different alleles.

arrows) and ankles (Figure E, arrows). X-ray showed segmented calcification of his Achilles tendons (Figure F, arrows).

A laboratory examination revealed liver dysfunction (aspartate aminotransferase 90 IU/L; normal 13-30 U/L, alanine aminotransferase 121 IU/L; normal 10-42 U/L), hypertriglyceridemia (triglyceride 411 mg/dL; normal 40-150 U/L) and diabetes mellitus (fasting blood glucose 179 mg/dL; normal 70-110 mg/dL, hemoglobin A1c of national glycohemoglobin standardization 6.9%; normal 4.6-6.2%). Dual-energy X-ray absorptiometry revealed a low bone density (cancellous bone mineral content of the 2nd, 3rd and 4th lumbar vertebrae: 0.822 g/cm^2; normal 0.895-1.137 g/cm^2, T-score -1.6; normal > -1.0).

Based on the diagnostic criteria (3), he was suspected of having WS. After obtaining his written informed consent, a

Table. Clinical Characteristics of Japanese Werner Syndrome Patients.

	Honjo et al. (2008)	Takemoto et al. (2013)		Present case (2018)
	Genetically confirmed (n=7)	Genetically confirmed (n=47)	Clinically diagnosed (n=146)	
Age	42.9% in 50s, 42.9% in 40s, 14.3% in 30s	62.7% in 60s, 22.7% in 50s, 10.8% in 40s, 1.1% in 30s, and 0.5% in 20s		38
Gender	Male 3, Female 4	Male 82, Female 93, unknown 18		Male
Height (cm)	n.m.	Male 158.3±8.6, Female 148.5±8.6		162.0
Body weight (kg)	n.m.	Male 45.3±8.3, Female 37.7±8.3		50.0
Gene mutations	c.3139-1G>C/ c.3139-1G>C	n.m.	n.m.	c.1720+1G>A/ c.3139-1G>C
Clinical symptoms				
Gray hair, loss of hair	100%	97.9%	98.2%	+
Bird-like face	n.m.	93.3%	97.2%	+
Bilateral cataract	100%	86.0%	75.5%	+
Abnormality of the voice	85.70%	82.2%	91.3%	+
Skin atrophy	100%	93.2%	99.0%	+
Skin ulcer	71.40%	86.3%	88.5%	+
Clavus or callus	n.m.	90.4%	92.4%	+
Flat foot	n.m.	94.6%	84.3%	+
Family history				
Consanguinity	n.m.	35.3%	40.6%	-
Parenthood	n.m.	34.4%	34.4%	-
Complications				
Diabetes mellitus	n.m.	55.8%	70.5%	+
Hypertension	n.m.	30.9%	37.0%	+
Dyslipidemia	n.m.	85.4%	60.7%	+
LDL-C<140	n.m.	50.0%	43.6%	-
HDL-C<40	n.m.	17.2%	25.9%	+
TG<150	n.m.	59.3%	55.9%	+
Fatty liver	n.m.	50.0%	42.1%	+
Cerebral hemorrhage	n.m.	2.5%	1.1%	-
Cerebral infarction	n.m.	2.4%	4.4%	-
Coronary heart disease	n.m.	11.1%	16.1%	-
Arteriosclerosis obliterans	n.m.	21.6%	25.8%	-
Malignant tumors	n.m.	44.4%	40.2%	-
Osteoporosis	n.m.	60.7%	66.4%	+
Markers for ectopic calcification (normal range)				
Serum calcium (mg/dL) (8.5-10.0)	9.3±0.28	n.m.	n.m.	9.9
Serum inorganic phosphate (mg/dL) (2.5-4.5)	3.4±0.40	n.m.	n.m.	3.1
Serum intact-parathyroid hormone (PTH) (pg/mL) (10.0-65.0)	45±13.7	n.m.	n.m.	15
Serum 1,25-(OH)2 Vitamin D (mg/mL) (20-60)	53±10.8	n.m.	n.m.	n.e.
Serum bone-specific alkaline phosphatase (BAP) (U/L)	28.3±27.8	n.m.	n.m.	9.4
Ectopic calcification				
Elbow	85.70%	n.m	n.m.	+
Knee	85.70%	n.m.	n.m.	+
Ankle	85.70%	76.5%	83.6%	+
Pain				
Elbow	71.40%	n.m.	n.m.	+
Knee	57.10%	n.m.	n.m.	+
Ankle	71.40%	n.m.	n.m.	+

LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, n.m.: not mentioned, n.e.: not evaluated

direct DNA sequence analysis was performed for WRN using a blood sample obtained during admission, revealing a c.1720+1G>A substitution (2) in intron 14 and c.3139-1G>C substitution in intron 25 of human WRN (Figure H and I). Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) using *Bsr*I performed on

DNA samples of the patient and his mother (his father was already deceased) confirmed that the c.1720+1G>A substitution (2) was in the patient only (Figure J). Allele-specific PCR (AS-PCR) performed for c.3139-1G>C using a common forward primer and two sets of reverse primers confirmed that the mutation was in both the patient and his mother (Figure K, Supplementary table). Reverse transcription (RT)-PCR was performed on RNA samples from the patient's leukocytes using flanking primers for exons 13 and 15 and exons 25 and 27, confirming skips in exons 14 and 26 due to c.1720+1G>A (2) and c.3139-1G>C, respectively (Figure L and M, Supplementary table). We subsequently conducted PCR using primers with specific base sequences for exons 13 and 26 and exons 14 and 27, which confirmed skips in exons 14 and 26 in different alleles, indicating that c.1720+1G>A (2) and c.3139-1G>C exist on different alleles (Figure N and O, Supplementary table).

He was treated with cimetidine and minodoronic acid for the calcification of his Achilles tendons, atorvastatin for dyslipidemia, metformin for diabetic mellitus and salicylic acid and pregabalin for his whole-body pain at our outpatient clinic for five years after discharge. During this time, the calcification of his bilateral Achilles tendons progressed slightly (Figure G). However, cancer screening has shown no malignancy thus far (currently 43 years old).

Discussion

We encountered a Japanese WS patient with compound heterozygous mutations of the WRN: one is a novel heterogeneous c.1720+1G>A substitution, while the other is the most frequent heterogeneous c.3139-1G>C substitution among Japanese (Figure H-K) (2). Most of the WRN mutations were related to exon skipping and the loss of function, showing similar clinical characteristics (4). In the present case, we confirmed that the novel c.1720+1G>A (2) also resulted in exon 14 skipping (Figure L). A previous report showed that the heterozygous c.3139-1G>C mutation, accompanied by exon 26 skipping, reduces the expression of WRN protein and the activity of DNA helicase (5), suggesting that the heterozygous c.1720+1G>A mutation (2), accompanied by exon 14 skipping, might also be related to a reduction in the WRN protein expression and DNA helicase activity. Although many Japanese WS patients are born from a consanguineous marriage and are homozygous for c.3139-1G>C (6), the present patient was not born from consanguineous parents and was compound heterozygous (Figure A, N and O). Of note, there have been several reports indicating that the number of WS patients from non-consanguineous marriages has been gradually increasing (3, 7).

WS patients often suffer from skin atrophy and subcutaneous calcification, resulting in painful ulcers (8); age-related disorders, such as type 2 diabetes mellitus; hypertension, dyslipidemia, osteoporosis, cardiovascular disease and malignant tumor (3). Malignant tumors are a leading cause of death in WS patients (4), including thyroid neoplasms (16.1%), malignant melanoma (13.3%), soft tissue sarcomas (10.1%), hematologic/lymphoid neoplasms (9.3%) and osteosarcoma (7.7%) (9). Although the present case with a novel mutation (c.1720+1G>A) showed similar clinical characteristics to previous Japanese WS patients, such as age-related disorders and symptoms related to ectopic calcification (Table), he has not developed any malignant tumors thus far (currently 43 years old), suggesting that WS patients with this a compound heterozygous mutation have a different phenotype than others (2).

The authors state that they have no Conflict of Interest (COI).

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