Recent Trends in *WRN* Gene Mutation Patterns in Individuals with Werner Syndrome

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OBJECTIVES: To determine recent trends in mutation patterns in the *WRN* gene, which cause Werner syndrome (WS), a rare, inheritable progeroid syndrome in Japan.

DESIGN: Retrospective cohort.

SETTING: Longitudinal survey of WS and literature search for case reports.

PARTICIPANTS: Individuals whose genetic testing their facilities had requested between 2009 and October 2016 (N = 67).

MEASUREMENTS: A nationwide epidemiological study was conducted from 2009 to 2011 to improve understanding of the pathology of WS and develop therapeutic guidelines. Since 2009, Chiba University Hospital consecutively evaluated the *WRN* gene in 67 individuals throughout Japan who had requested genetic testing. A literature search was also conducted for case reports on Japanese WS reported since 1997.

RESULTS: A definitive diagnosis of WS was confirmed genetically in 50 of 67 participants. Through the literature search, 16 individuals diagnosed genetically with WS were identified. Of these 66 individuals with WS, 42 were homozygous for a *WRN* mutation, and 21 were compound heterozygotes. One novel mutant allele was identified in an individual with the compound heterozygous genotype. The proportion of compound heterozygotes (31.8%) was significantly greater than reported previously (14.2%), indicating that the incidence of consanguineous marriage of parents has decreased.

CONCLUSION: The increased frequency of individuals with WS with the compound heterozygous genotype is a recent trend in Japan. A long-term follow-up study on *WRN* homozygotes and compound heterozygotes will allow the relationship between *WRN* genotype and clinical severity of WS to be evaluated in the future. **J Am Geriatr Soc 65:1853–1856, 2017.**

Key words: Werner syndrome; Werner gene; gene mutation; ReqQ DNA helicase

Werner syndrome (WS), also known as adult progeroid syndrome, is an autosomal-recessive disorder caused by a mutation in the gene encoding the RecQ DNA helicase[1], with a high incidence in Japan[2]. In 2009, a nationwide study aimed at understanding the pathology and development of therapeutic guidelines for WS was launched as intractable disease research supported by Health, Labour and Welfare Sciences Research grants from the Ministry of Health. The study remains ongoing in a form of research on rare and intractable diseases. Since 2009, Chiba University Hospital has conducted genetic testing of WS as requested by facilities across Japan. A literature search was also conducted for cases of WS in Japanese individuals reported since 1997. A summary of the results of the genetic testing conducted and the literature search is reported.

MATERIALS AND METHODS

Subjects

Participants were 67 individuals whose genetic testing their facilities had requested between 2009 and October 2016. The present study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by our ethical committee before its inception. All participants understood the study aims and methods and provided written informed consent.

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Literature Search

A literature search was conducted using the medical online database (http://mol.medicalonline.jp/library/) for articles written in Japanese and the PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) archives with the following terms: "Werner syndrome" [All Fields] AND {Case Reports (ptyp) AND ["1996/01/01"(PDAT): "3000/12/31"(PDAT)]} from 1997. Multiple reports on the same individuals and those on individuals whose genetic testing the authors performed were excluded.

Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from anonymized blood samples collected using ethylenediaminetetraacetic acid 2Na (QIAamp DNA Blood Mini Kit, QIAGEN, Hilden, GERMANY). In the genetic testing, the exons were amplified individually, as described previously[3]. The amplified DNA fragments were subjected to direct sequencing for the analysis; 11 mutations common in Japanese subjects were examined. Additional genetic analysis was conducted in some participants.

Statistical Analysis

Comparisons between two groups were conducted using the chi-square test. $P < .05$ was considered statistically significant. Statistical analyses were performed using JMP Pr012 software (SAS Institute Japan, Tokyo, Japan).

RESULTS

Sixty-seven individuals were genotyped. Genotyping in these cases was requested from facilities in 26 of 46 prefectures in Japan. Internal medicine (32.8%) and dermatology (19.4%) departments frequently requested the genetic testing. WS was the genetically definitive diagnosis in 50 cases. The literature search identified 186 reports describing WS, and 49 of these were Japanese WS case reports from the medical online database. In these 49 reports, genotypes were reported in six individuals, three of which the authors had genetically diagnosed and excluded from the study. Of 151 reports of WS identified from PubMed, 51 were Japanese, and genotypes were reported in 15. Multiple reports of the same individuals and those of individuals in whom the authors had performed genetic testing were excluded, leaving 13 individuals for evaluation. In total, 16 individuals genetically diagnosed with WS were identified from the literature.

Table 1 shows the breakdown of the frequency of genotypes. There were 41 homozygotic, 21 compound heterozygotic, and four heterozygotic (only one allele has a mutation) participants. The frequency of compound heterozygosity was significantly higher (31.8%) than reported previously (14.2%) ($P = .02$) (Table 1). Sixteen individuals who underwent genotyping in Chiba University Hospital were free of the mutations. One novel allele mutation, such as the type 6 mutation (c.1105C>T)/c.2772delA (Table 1), was identified in 18 compound heterozygotes who also underwent genotyping in our facility.

Table 1. Breakdown of the Frequency of *WRN* Genotypes in This Report Compared to Previous Report

Mutation	Participants from This Report	Participants from Previous Reports	*P*-Value
	n (%)		
Homozygote	42 (63.6)	41 (73.2)	.19
Mut 1/1	0 (0)	1 (1.8)	.80
Mut 4/4	31 (47.0)	26 (46.4)	.95
Mut 5/5	0 (0)	1 (1.8)	.28
Mut 6/6	9 (15.1)	9 (16.1	.71
Mut 7/7	1 (1.5)	1 (1.8)	.91
Mut 8/8	0 (0)	1 (1.8)	.28
Mut 9/9	0 (0)	1 (1.8)	.28
Mut 10/10	0 (0)	1 (1.8)	.28
Compound heterozygote	21 (31.8)	8 (14.2)	.02
Mut 4/6	6 (9.1)	3 (5.4)	.43
Mut 4/7	5 (7.6)	0 (0)	.04
Mut 1/4	2 (3.0)	5 (8.9)	.16
Mut 4/11	3 (4.5)	0 (0)	.11
Mut 4/ IVS 14+1 G>A	1 (1.5)	0 (0)	.36
Mut 1/11	1 (1.5)	0 (0)	.36
Mut 1/6	1 (1.5)	0 (0)	.36
Mut 1/ 3030–3033delAACG	1 (1.5)	0 (0)	.36
Mut 6/ 2772delA (novel mutation)	1 (1.5)	0 (0)	.36
Heterozygote	4 (6.1)	7 (12.5)	.22
1/?	2 (3.0)	2 (3.6)	.87
4/?	2 (3.0)	4 (7.1)	.30
6/?	0 (0)	1 (1.8)	.28
Total	66	56	

Frequency of each genotype in the study was compared with that in a previous report[5].

Mut 1: c.3913C>T, Mut 4: c.3139–1G>C, Mut 5: c.3915dupA, Mut 6: c.1105C>T, Mut 7: c.3446delA, Mut 8: c.3460–7T>A, Mut 9: c.1389T>A, Mut 10: c.502_503delAA, Mut11: c.2959C>T.

The genotype–phenotype relationship between homozygotes and compound heterozygotes was also analyzed. In this analysis, the clinical signs and symptoms listed in the diagnostic criteria were compared. Twenty-six homozygotic and 14 compound heterozygotic individuals aged 40 and older were selected, because most clinical symptoms do not appear until the age of 40. Parental consanguinity was observed frequently in homozygotes but not in compound heterozygotes (Table 2). There were no differences in major clinical signs and symptoms between the two groups.

DISCUSSION

Previous studies in Japan in 1978 and 1981 revealed that WS is inherited as an autosomal-recessive trait[4]. Approximately 1,200 cases of WS have been reported worldwide. The genetic epidemiology of the Japanese population with WS was reported previously in 1997, and approximately 1,000 cases have been found in Japan[5]. According to a report of a genetic assessment of 1,000 general inhabitants in Kanagawa Prefecture, six residents had heterozygous mutations in the *WRN* gene, which would mean that,

Table 2. Genotype–Phenotype Relationship Between Homozygotes and Compound Heterozygotes

	Homozygote		Compound Heterozygote		
	Positive Signs	Negative Signs	Positive Signs	Negative Signs	
	n				*P*-Value
Cardinal signs and symptoms					
Progeroid changes of hair	22	1	12	1	.68
Cataract	26	0	14	0	N/A
Skin changes, intractable skin ulcers	25	1	14	0	.35
Soft-tissue calcification	18	0	11	0	N/A
Bird-like face	19	3	9	1	.77
Abnormal voice	16	5	9	1	.34
Other signs and symptoms					
Abnormal glucose or lipid metabolism	16	11	12	2	.07
Deformation and abnormality of the bone	13	3	9	0	.09
Malignant tumors	7	14	1	9	.14
Parental consanguinity	14	11	0	12	.001
Premature atherosclerosis	3	11	4	4	.17
Hypogonadism	1	8	1	4	.65
Short stature and low body weight	13	13	6	6	>.99

Clinical signs and symptoms, which were listed in the diagnostic criteria, of 26 homozygotes and 14 compound heterozygotes aged 40 and older were compared.

The number of patients is indicated. For instance, 22 of 26 homozygotic participants had progeroid changes of the hair, and one had no change. The clinical findings have not always been described well in published case reports. Therefore, the total number of participants with each clinical sign differed.

N/A=not available.

mathematically, approximately 23 homozygote individuals would be born every year[2].

There are 83 types of *WRN* gene mutations, including nonsense, splicing, and frameshift mutations, which have been reported recently[1,6,7]. In Japan, the type 4 mutation (mut4), in which G, the base immediately preceding exon 26, is mutated to C (c. 3139–1G>C) is the most common (50.4%), followed by the type 6 mutation (mut6) (c.1105C>T) (17.5%)[5]. These mutation names (mut4, mut6) are used in Japan. The frequency of mut4 (48%) was also highest in the 50 cases in which the present genetic testing led to the definitive diagnosis—similar to the percentage in the previous report. Such a development due to homozygosity is seen frequently in consanguineous marriages. In nonconsanguineous marriage, WS develops because of compound heterozygosity, such as mut4 (c.3139–1G>C)/mut6 (c.1105C>T). According to a previous report, the rate of development of WS from consanguineous marriage was 70%, whereas that from compound heterozygosity was as rare as 14.2%[5]. According to a Japanese nationwide epidemiological survey of WS conducted in 2009, the incidence from consanguineous marriage was 43%, which was lower than in the previous report[7]. The increase in incidence from compound heterozygosity to 31.8% in the present examination reflects this, supporting that development of WS from nonconsanguineous marriage is increasing in Japan. Greater numbers of compound heterozygotes indicates that people with the *WRN* heterozygote mutation have spread widely throughout Japan, probably because of the development of transportation network. The current study also provided information on how rare diseases (in this case, WS) spread genetically on large islands such as Japan. Autozygosity, the genomic signature of consanguinity, has declined because of globalization and urbanization[8]. In addition to these influences of demographic factors on inherited rare diseases, recent improvement in genomic sequencing technique might also have affected the results, because there were more heterozygotes in previous studies (7 of 56 cases) than in the current study (4 of 66 cases), although the difference was not statistically significant (Table 1).

Few reports have indicated relationships between WS genotype and its clinical severity[9]. The current study attempted to determine the difference in phenotypes between homozygotes and compound heterozygotes. Although it was not possible to detect any differences in phenotypes, further analyses should be performed before any conclusions are drawn. The clinical findings of these individuals have not always been well described in published case reports, and some characteristic phenotypes in each genotype may appear later in life. For that reason, whether there is a difference in phenotype between homozygotes and compound heterozygotes is a matter for future examination and will require long-term follow-up. Therefore, a registry for Japanese with WS has been started, which The Japan Agency for Medical Research and Development has supported (http://www.m.chiba-u.jp/class/clin-cellbiol/werner/index.html).

This examination allowed one novel gene mutation to be identified. Relationships between these novel mutations and phenotypes are interesting. In addition, of the cases suspected clinically to be progeria, 16 had no mutation in the *WRN* gene. For these cases, additional genes, including the *LMNA* gene[10], will be analyzed, which also may lead to identification of a novel type of progeria.

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