J Bone Miner Metab (2015) 33:694–700
DOI 10.1007/s00774-014-0636-0

ORIGINAL ARTICLE

A missense single nucleotide polymorphism, V114I of the Werner syndrome gene, is associated with risk of osteoporosis and femoral fracture in the Japanese population

Heying Zhou · Seijiro Mori · Masashi Tanaka · Motoji Sawabe · Tomio Arai · Masaaki Muramatsu · Makiko Naka Mieno · Shoji Shinkai · Yoshiji Yamada · Motohiko Miyachi · Haruka Murakami · Kiyoshi Sanada · Hideki Ito

Received: 16 June 2014 / Accepted: 13 September 2014 / Published online: 31 January 2015

Abstract Werner syndrome is a rare autosomal recessive disorder caused by mutations in the human *WRN* gene and characterized by the early onset of normal aging symptoms. Given that patients with this disease exhibit osteoporosis, the present study aimed to determine whether the *WRN* gene contributes to the etiology of osteoporosis. A genetic association study of eight non-synonymous polymorphisms in the *WRN* gene and the incidence of femoral fracture was undertaken in 1,632 consecutive Japanese autopsies in which 140 patients had experienced the fracture during their lifetime. The results were validated in 251 unrelated postmenopausal Japanese women with osteoporosis and 269 non-institutionalized, community-dwelling Japanese adults. A statistically significant association was observed between rs2230009 (c.340G > A)—which results in a Val to Ile substitution—and fracture risk; the incidence of femoral fracture increased dose-dependently with the number of A alleles ($p = 0.0120$). Femoral neck bone and whole bone densities were lower among postmenopausal women with osteoporosis and community-dwelling adults, respectively, if they were of the AG instead of the GG genotype. The results suggest that Japanese subjects bearing at least one A allele of rs2230009 of the *WRN* gene are at a significantly higher risk of femoral fracture, possibly due to decreased bone density.

H. Zhou · S. Mori (✉)
Center for Promotion of Clinical Investigation, Tokyo Metropolitan Geriatric Hospital, 35-2 Sakae, Itabashi, Tokyo 173-0015, Japan
e-mail: mori_seijiro@tmghig.jp

M. Tanaka
Department of Genomics for Longevity and Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

M. Sawabe
Section of Molecular Pathology, Graduate School of Health Care Sciences, Tokyo Medical and Dental University, Tokyo, Japan

T. Arai
Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

M. Muramatsu
Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

M. N. Mieno
Department of Medical Informatics, Center for Information, Jichi Medical University, Shimotsuke, Tochigi, Japan

S. Shinkai
Research Team for Social Participation and Community Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Y. Yamada
Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Mie, Japan

M. Miyachi · H. Murakami
Department of Health Promotion and Exercise, National Institute of Health and Nutrition, Tokyo, Japan

K. Sanada
Faculty of Sport and Health Science, Ritsumeikan University, Kita, Shiga, Japan

H. Ito
President, Local Independent Administrative Agency, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan

Keywords Femoral fracture · Osteoporosis · Single nucleotide polymorphism · Werner syndrome

Introduction

Werner syndrome (WS) is a premature aging disease characterized by the early onset of normal aging symptoms, including atherosclerosis, cancer, non-insulin-dependent diabetes mellitus, ocular cataracts, graying and loss of hair, and osteoporosis [1, 2]. Mutations in the human *WRN* gene, which encodes a member of the RecQ family of DNA helicases, give rise to this rare autosomal recessive genetic disorder [3]. It has been reported that cells derived from patients with a *WRN* mutation show increased genomic instability, manifested as chromosomal alterations [4, 5]; WRN protein participates in several important DNA metabolic pathways, including DNA replication, recombination, and telomere maintenance, that together preserve genomic integrity [6, 7]. Although these experimental observations highlight the important link between defective DNA repair mechanisms and aging or cancer, the contribution of *WRN* mutations to these processes remains an open question.

Several recent studies have suggested an association between single nucleotide polymorphisms (SNPs) in the *WRN* gene and a variety of disease conditions, including non-Hodgkin lymphoma [8, 9], breast cancer [10, 11], chronic kidney disease [12], myocardial infarction [13, 14], bone and soft tissue sarcomas [15], the development of benzene hematotoxicity [16], and decreased bone density [17]. One conjecture that can be made based on these reports is that partial alterations in *WRN* gene function lead to the development of diseases that are commonly seen in the elderly, since the complete loss of gene function by homozygous *WRN* mutations results in a premature aging syndrome. To test this hypothesis, eight non-synonymous SNPs in the *WRN* gene—rs2230009 (V114I), rs17847577 (R369X), rs113811718 (E510D), rs77969734 (L628 V), rs34560788 (R711 W), rs1801195 (F1074L), 201107091 (A1260T), and rs1346044 (C1367R)—were investigated for a possible genetic association with osteoporosis, an aging-related condition.

Materials and methods

Subjects

The subjects consisted of autopsy cases, patients with osteoporosis, and community-dwelling adults from the Japanese population.

The autopsies were performed consecutively at Tokyo Metropolitan Geriatric Hospital between 1995 and 2011, and comprised 924 males and 708 females with a mean age of 81 years. None of the female subjects had undergone hormone replacement therapy. The presence or absence of any diseases was determined by thorough examination of the autopsy report. Most major pathological diseases were included in the database. The presence or absence of a femoral fracture during each subject's lifetime was determined by thorough examination of the clinical record. Traumatic fractures due to traffic accidents, as well as apparent pathological fractures caused by metastatic cancers, were excluded from the assessment. In total, 140 patients (39 male, 101 female) with a femoral fracture were identified.

Osteoporosis patients comprised 251 unrelated postmenopausal women (with a mean age of 73 years) who visited outpatient clinics of Tokyo Metropolitan Geriatric Hospital between 2006 and 2011. Before their first visit to the clinics, some patients had already been treated with drugs commonly prescribed for osteoporosis in Japan, including bisphosphonates ($n = 125$), active vitamin D ($n = 93$), the selective estrogen receptor modulator raloxifene ($n = 18$), and vitamin K2, i.e., menatetrenone ($n = 15$), either alone or in different combinations. The diagnosis of osteoporosis was made according to the Japanese diagnostic criteria for primary osteoporosis [18]. Individuals with disorders known to cause abnormalities in bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, and thyroid, parathyroid, or other endocrinological diseases, were excluded from the study.

The community-dwelling adults included 269 non-institutionalized subjects (133 men and 136 women, with a mean age of 75 years) who participated in a comprehensive health examination in September 2012 in Hatoyama (Saitama Prefecture). All participants read and signed informed consent forms, which were approved along with the study protocol by the ethics committee of Tokyo Metropolitan Geriatric Hospital and the Clinical Research Ethics Committee of the Tokyo Metropolitan Institute of Gerontology. For autopsy cases, written, informed consent, including for the use of extracted DNA in medical and genetic studies, was obtained from the family prior to the autopsy.

Genotyping

To determine the functional relationship between *WRN* gene polymorphisms and osteoporosis, non-synonymous and putative functional SNPs were examined. The Illumina Infinium HumanExome BeadChip v1.1 [19] contained a set of 42 non-synonymous and two synonymous SNPs in the *WRN* gene, of which eight were polymorphic in the study population: rs2230009 (V114I), rs17847577 (R369X), rs113811718 (E510D), rs77969734 (L628 V), rs34560788 (R711 W), rs1801195 (F1074L), rs201107091 (A1260T), and rs1346044 (C1367R). These were evaluated for their

association with the incidence of femoral fracture in autopsy cases. Total genomic DNA was extracted from the renal cortex using phenol/chloroform according to the standard protocol. Study samples were processed on the HumanExome BeadChip v1.1 (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's recommendations. The genotyping success rate was 100 % for rs2230009, rs17847577, rs113811718, rs77969734, rs34560788, and 201107091; 99.8 % for rs1801195; and 99.9 % for rs1346044.

For validation, genotyping of rs2230009 was carried out using a real-time PCR system (StepOnePlus; Applied Biosystems, Foster City, CA, USA) with the TaqMan SNP genotyping assay method [20] using ID C_15851779_10 (Applied Biosystems); the genotyping success rate was 100 %.

Measurement of body composition

Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, and body mass index (BMI) was calculated by dividing the body weight (kg) by the square of height (m^2).

For community-dwelling adults, whole body bone mineral density (BMD) and fat-free mass (FFM) were obtained by dual-energy X-ray absorptiometry (DXA) (Hologic QDR-4500A scanner; Hologic, Waltham, MA, USA) using a previously described method [20]. The whole body FFM was divided into several regions, i.e., arms, legs, trunk, and head, to estimate appendicular FFM (sum of arms and legs) and relative skeletal muscle index (RSMI), which was calculated as the ratio of appendicular FFM to the square of height. Body compositions were analyzed using manual DXA analysis software, version 11.2:3 (Hologic).

For osteoporosis patients, lumbar spine (anteroposterior, L2–L4) and femoral neck BMD, as well as appendicular FFM, were determined by DXA (Model DPX-L, software version 3.2; Lunar Radiation, Madison, WI, USA) using a previously described method [21]. Fractured lumbar vertebrae were excluded from BMD calculations.

Measurement of serum 25-hydroxyvitamin D (25OHD)

Blood samples were collected in a fasting state and sitting position and analyzed by a single laboratory (Special Reference Laboratories, Tokyo, Japan). Serum 25OHD concentration was measured using a RIT 2 kit (DiaSorin, Stillwater, MN, USA), which is based on an antibody specific to 25OHD; using this method, the coefficient of variation was <1 %.

Statistical analysis

The allele frequency was calculated, and a χ^2 test was used to determine the deviation of the genotype distribution from Hardy–Weinberg equilibrium. Analysis of association was based on linear or logistic regression according to the response variable (quantitative or binary disease status, respectively). Multiple logistic regression analysis was conducted to estimate the odds ratio (OR) and 95 % confidence interval (CI) for the association between femoral fracture risk and genotype, sex, and age. The Cochran-Armitage proportion trend test was used to identify changes in fracture incidence with respect to the number of risk alleles. For a quantitative analysis, linear regression—adjusting for sex and age as covariates—was conducted to estimate the difference and 95 % CI. All statistical analyses except for the linear regression were performed using SAS ver.9.2 (SAS Institute Inc., Cary, NC, USA). Linear regression analyses were performed using SNP Stats [22]. A two-sided p value of less than 0.05 was considered statistically significant.

Results

To investigate the association between eight non-synonymous SNPs in the *WRN* gene and the incidence of femoral fracture, 1,632 consecutive autopsies were studied; 140 were of individuals who experienced femoral fracture during their lifetime. The minor allele frequency (MAF) of four SNPs (rs17847577, rs113811718, rs34560788, and rs20110791), was less than 0.01, and these were excluded from subsequent analyses since a precise statistical evaluation was not possible. Of the remaining SNPs, MAFs of rs2230009, rs77969734, rs1801195, and rs1346044 were 0.018, 0.017, 0.42, and 0.067, respectively, and their genotype frequency distributions were in Hardy–Weinberg equilibrium.

The relationship between SNP genotypes and incidence of femoral fracture is shown in Table 1. A statistically significant association was observed between rs2230009 (c.340G > A, p.V114I) and fracture risk; the incidence of femoral fracture increased dose-dependently with the number of A alleles ($p = 0.0120$), as assessed by the exact Cochran-Armitage trend test. Applying a dominant model, the logistic regression analysis revealed an OR of 2.528 (95 % CI: 1.194–5.350; $p = 0.0154$) for rs2230009 after adjusting for sex and age at autopsy (Table 2). Prevalence of femoral fracture was negatively associated with that of left ventricular hypertrophy ($p = 0.0089$), colorectal cancer ($p = 0.0449$), pulmonary cancer ($p = 0.0293$), and positively with Parkinson's disease ($p = 0.0060$); however, none of these diseases were associated with the rs2230009 genotype. There was no association between femoral fracture and renal/parathyroidal diseases, which sometimes affect calcium metabolism (data not shown).

Table 1 Prevalence of femoral fracture in 1,632 consecutive Japanese autopsies with different genotypes

SNP*	Genotype	Femoral fracture†	*N*	*p* value
rs2230009	AA	1 (50.0)	2	0.0120‡
	AG	9 (16.4)	55	
	GG	130 (8.3)	1575	
rs77969734	CC	133 (8.4)	1578	0.3043
	CG	7 (13.5)	52	
	GG	0 (0)	2	
rs1801195	GG	25 (8.4)	299	0.4713
	GT	62 (7.9)	781	
	TT	52 (9.5)	548	
rs1346044	CC	0 (0)	6	0.2409
	CT	14 (6.8)	205	
	TT	126 (8.9)	1419	

* Single nucleotide polymorphisms in the *WRN* gene

† Number (percent) of subjects is presented

‡ Significant ($p < 0.05$), as assessed by the exact Cochran-Armitage trend test

Table 2 Multiple logistic regression analysis of the association between the prevalence of femoral fracture and genotype, sex, and age

Factors	OR (95 % CI)	*p* value
rs2230009, AA/AG vs. GG	2.528 (1.194–5.350)	0.0154*
Sex, women vs. men	2.983 (1.988–4.776)	<0.0001*
Age at autopsy, 10 years older	1.746 (1.396–2.185)	<0.0001*

OR odds ratio, *CI* confidence interval

* Significant ($p < 0.05$)

To eliminate possible statistical error, we performed replication analyses using secondary cohorts to confirm the association between rs2230009 and osteoporosis, since none of the associations remained positive after Bonferroni correction. To validate the observed association, 251 unrelated postmenopausal Japanese women with osteoporosis and 269 non-institutionalized, community-dwelling Japanese adults were evaluated. The characteristics of patients with postmenopausal osteoporosis are shown in Table 3. There were no significant differences in age, weight, height, BMI, appendicular FFM, RSMI, and serum levels of calcium or 25OHD between GG and AG genotypes. In contrast, femoral neck BMD was lower in patients with the AG than with the GG genotype; a linear regression analysis showed the difference to be −0.04 g/cm^2 (95 % CI −0.08–0.00; $p = 0.041$) after adjusting for age. Lumbar BMD was also lower in patients with the AG than with the GG genotype; however, the difference was not statistically significant ($p = 0.068$).

As shown in Table 4, whole body BMD was significantly lower in subjects with the AG than with the GG genotype among 269 community-dwelling adults when analyzed as a whole; the difference was −0.07 g/cm^2 (95 % CI −0.12 to −0.02; $p = 0.0079$), as estimated by linear regression analysis after adjusting for sex and age. A difference in the genotype-dependent whole body BMD was also found ($p = 0.016$) when only male subjects were analyzed; among female subjects, whole body BMD was lower in AG than in GG allele carriers, but the difference was not statistically significant ($p = 0.20$). As expected, no differences were observed in appendicular FFM and RSMI between GG and AG genotypes.

Table 3 Clinical characteristics of 251 postmenopausal women with osteoporosis stratified by rs2230009 genotype

	GG ($n = 236$)		AG ($n = 15$)		Difference (95 % CI)	*p* value
	Mean	SD	Mean	SD		
Age (years)	70.9	8.09	71.7	6.83	0.76 (−3.43–4.94)	0.724
Weight (kg)	48.0	6.81	44.7	5.00	−3.33 (−6.97–0.32)	0.074
Height (cm)	150	11.4	140	38.5	−11.20 (−32.6–10.1)	0.279
BMI (kg/m^2)	21.0	2.88	20.1	2.51	−0.92 (−2.46–0.61)	0.240
Appendicular FFM (kg)	12.7	1.52	12.4	1.48	−0.24 (−1.18–0.71)	0.620
RSMI (kg/m^2)	5.51	0.54	5.55	0.52	0.03 (−0.31–0.37)	0.850
Lumbar spine BMD (g/cm^2)	0.79	0.14	0.73	0.17	−0.07 (−0.14–0.00)	0.068
Femoral neck BMD (g/cm^2)	0.63	0.08	0.59	0.08	−0.04 (−0.08–0.00)	0.041*
Serum calcium level (mg/dl)	9.65	0.41	9.53	0.31	−0.12 (−0.33–0.09)	0.270
Serum 25OHD level (ng/ml)	21.5	6.45	19.4	5.15	−2.02 (−5.35–1.30)	0.230
Oral bisphosphonate use, *n* (%)	116 (49)		9 (60)			
Active vitamin D use, *n* (%)	87 (37)		6 (40)			
Raloxifene use, *n* (%)	17 (7)		1 (7)			
Menatetrenone use, *n* (%)	15 (6)		0 (0)			

BMD bone mineral density, *BMI* body mass index, *CI* confidence interval, *FFM* fat-free mass, *25OHD* 25-hydroxyvitamin D, *RSMI* relative skeletal muscle index, *SD* standard deviation

*Significant ($p < 0.05$), as assessed by linear regression analysis after adjusting for age

Table 4 Body composition data for 269 community-dwelling Japanese adults stratified by rs2230009 genotype

	GG		AG		Difference (95 % CI)	*p* value
	Mean	SD	Mean	SD		
Total						
n	259		10			
Whole body BMD (g/cm^2)	0.87	0.10	0.80	0.06	−0.07 (−0.12 to −0.02)	0.0079*
Appendicular FFM (kg)	18.1	3.72	17.8	3.30	−0.48 (−1.89 to 0.94)	0.52
RSMI (kg/m^2)	7.40	0.97	7.20	0.85	−0.21 (−0.71 to 0.28)	0.40
Female						
n	131		5			
Whole body BMD (g/cm^2)	0.81	0.08	0.76	0.03	−0.05 (−0.12 to 0.03)	0.20
Appendicular FFM (kg)	15.2	1.82	15.4	1.39	0.06 (−1.54 to 1.67)	0.94
RSMI (kg/m^2)	6.83	0.72	6.90	0.90	0.10 (−0.55 to 0.76)	0.76
Male						
n	128		5			
Whole body BMD (g/cm^2)	0.93	0.08	0.84	0.05	−0.09 (−0.16 to −0.02)	0.016*
Appendicular FFM (kg)	21.1	2.68	20.1	2.98	−0.89 (−3.23 to 1.45)	0.46
RSMI (kg/m^2)	7.98	0.85	7.49	0.77	−0.47 (−1.21 to 0.28)	0.22

BMD bone mineral density, *CI* confidence interval, *FFM* fat-free mass, *RSMI* relative skeletal muscle index, *SD* standard deviation

*Significant ($p < 0.05$), as assessed by linear regression analysis after adjusting for sex and age

Discussion

In the present study, a statistically significant association between rs2230009 (c.340G > A, p.V114I) and the incidence of femoral fracture was found among autopsy cases in the Japanese population, such that the presence of at least one copy of the A allele increased fracture risk (Tables 1, 2). However, it should be noted that the MAF for rs2230009 was 0.018; hence, the number of femoral fracture cases with the risk allele was very small; there were only one and nine cases for the AA and AG genotypes, respectively (Table 1). This finding was validated by genotyping rs2230009 in patients with postmenopausal osteoporosis: femoral neck BMD was lower in A allele than in homozygous G allele carriers (Table 3). Likewise, among community-dwelling adults, whole body BMD was lower in A allele than in homozygous G allele carriers (Table 4). To our knowledge, this is the first study to investigate the association between genetic polymorphisms in *WRN* and the incidence of femoral fracture. The results suggest that Japanese subjects bearing at least one A allele of rs2230009 have a significantly higher risk of femoral fracture, possibly due to decreased bone density.

It is important to know whether the higher risk of femoral fracture in subjects with the rs2230009 A allele is a direct effect of this polymorphism for bone or an indirect effect through dysfunction of other organs or tissues. To differentiate the effects, we examined previous histories and pathological findings of subjects; however, we found that there was no correlation with diseases that could mediate the association between this polymorphism and femoral fracture. This finding suggests a direct association between them.

WS patients exhibit osteoporosis [23–25] with possible impaired osteoblastic bone formation [26], but normal osteoclastic bone resorption [27, 28]. A target of the WRN protein is telomeric DNA [7], but long telomeres and abundant telomerase in mice minimize the need for *WRN*, and thus *WRN* knockout mice are relatively healthy. However, in a model of accelerated aging that combined a *WRN* mutation with the shortened telomeres of telomerase (*TERC*) knockout mice, the simultaneous loss of *WRN* and *TERC* genes produced a low bone mass phenotype, and age-related osteoporosis resulted from impaired osteoblast differentiation [29]. Although there is no evidence to date for the expression and function of the WRN protein in human bone cells including osteoblasts, this, along with a subsequent report [30], suggests that defective osteoblast differentiation due to telomere dysfunction is an important cellular mechanism that could partly explain the early onset of osteoporosis in WS patients. Thus, it is possible that partial alterations of *WRN* gene function play a role in the development of osteoporosis during the normal course of human aging. WRN protein has different functional domains, including exonuclease, helicase, RecQ C-terminal, and helicase and RNaseD C-terminal domains, as well as a nuclear localization signal [31]. The exonuclease domain is encoded by exons 4–7 of the *WRN* gene, and rs2230009 is located in exon 4; however, it remains to be determined whether a Val → Ile substitution at residue 114 or a G → A transition at nucleotide position 340 can alter WRN protein function.

A G574R missense mutation in the *WRN* gene was recently identified in a WS patient [32]; the mutation was found to inhibit ATP binding, leading to a decrease in helicase activity, while preserving the exonuclease activity. It

was concluded that impaired helicase activity may have caused defects in replication and other DNA metabolic processes in the patient, contributing in turn to the WS phenotype. In contrast to typical manifestations of WS, the G574R patient reportedly had normal stature, leading to the suggestion that the stunted growth normally associated with WS may not be due to a defective helicase. The present finding that the V114I missense polymorphism in the *WRN* gene, which alters an amino acid residue in the exonuclease domain, was associated with osteoporosis also supports the conjecture that the loss of exonuclease activity is responsible for skeletal manifestations in WS patients, including a short stature and osteoporosis.

An association of rs1346044 (C1367R) with bone density in the lumbar spine as well as the whole body was reported among unrelated Japanese postmenopausal women [17]. However, the present study did not find a significant association between this SNP genotype and the incidence of femoral fracture among autopsy cases (Table 1). Although the reason for this discrepancy is unclear at present, it may arise from differences in methodology for the evaluation of bone fragility (i.e., by measuring bone density as compared to calculating fracture incidence, as was done in the current study).

To date, at least 17 genome-wide association studies (GWASs) have been performed, and meta-analysis of these studies identified 56 BMD-associated loci of genome-wide significance [33]. However, the previous GWASs did not identify the *WRN* gene as a major candidate gene for osteoporosis. The disparity in results may be due to the fact that the MAF for rs2230009 is 0.018, which is too low to be analyzed by GWASs that detect the association among common sequence variants (MAF > 0.05). Another explanation is ethnic differences, since most of the subjects in the reported GWASs were from Northern European descent. It is well known that reported WS cases are mostly Japanese, and the disease is extremely rare in other countries compared to Japan. Therefore, there may be some ethnic differences in the mode of phenotype expression because of an alteration in *WRN* gene function.

The mechanism underlying the association between the genetic polymorphism in the *WRN* gene and fracture risk remains to be elucidated. Future studies will include not only a functional analysis of the WRN protein in the context of bone metabolism, but also genetic analyses exploring the effect of the polymorphism on the WRN protein. However, the possibility cannot be ruled out that the SNP marker may itself be in linkage disequilibrium with other unmeasured and functional variants at or near the *WRN* gene, which compose the true mechanistic basis for the observed association. The rs2230009 SNP may nonetheless serve as a useful polymorphic marker for identifying at-risk individuals and implementing preventive measures against osteoporosis. The results of this study warrant further replication with larger size samples.

Acknowledgments This work was supported by a grant (no. 09042037) from the Leading Project for Personalized Medicine of the Ministry of Education, Culture, Sports, Science and Technology of Japan (to S.M.); Grants-in-Aid for Scientific Research (nos. 22240072, 21390459, and 21590411 [to M.T.], 18209023, 18018021, 19659149, and 24590746 [to YY], and 23240089 [to MM]); a Grant-in-Aid for the Global COE (Sport Sciences for the Promotion of Active Life to Waseda University) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to MT); a Grant-in-Aid for Scientific Research (to MM) and Grants-in-Aid (H23-016, H23-119, and H24-005) for Research on Intractable Diseases (Mitochondrial Disease) (to MT) from the Ministry of Health, Labour and Welfare of Japan; grants for scientific research from Mitsui Sumitomo Insurance Welfare Foundation (to SM); the Pfizer Academic Contribution Fund (to SM); Takeda Science Foundation (to MT); the Smoking Research Foundation (to TA and MS); and the Mie Medical Valley Project (to YY).

Conflict of interest Heying Zhou, Seijiro Mori, Masashi Tanaka, Motoji Sawabe, Tomio Arai, Masaaki Muramatsu, Makiko Naka Mieno, Shoji Shinkai, Yoshiji Yamada, Motohiko Miyachi, Haruka Murakami, Kiyoshi Sanada, and Hideki Ito declare that they have no conflict of interest.

References

1. Epstein CJ, Martin GM, Schultz AL, Motulsky AG (1966) Werner's syndrome, a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Medicine 45:177–221
2. Takemoto M, Mori S, Kuzuya M, Yoshimoto S, Shimamoto A, Igarashi M, Tanaka Y, Miki T, Yokote K (2013) Diagnostic criteria for Werner syndrome based on Japanese nationwide epidemiological survey. Geriatr Gerontol Int 13:475–481
3. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD (1996) Positional cloning of the Werner's syndrome gene. Science 272:258–262
4. Salk D, Au K, Hoehn H, Martin GM (1981) Cytogenetics of Werner's syndrome cultured skin fibroblasts: variegated translocation mosaicism. Cytogenet Cell Genet 30:92–107
5. Ogburn CE, Oshima J, Poot M, Chen R, Hunt KE, Gollahon KA, Rabinovitch PS, Martin GM (1997) An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type to homozygous mutants. Hum Genet 101:121–125
6. Rossi ML, Ghosh AK, Bohr VA (2010) Roles of Werner syndrome protein in protection of genome integrity. DNA Repair 9:331–344
7. Crabbe L, Jauch A, Naeger CM, Holtgreve-Grez H, Karlseder J (2007) Telomere dysfunction as a cause of genomic instability in Werner syndrome. Proc Natl Acad Sci USA 104:2205–2210
8. Shen M, Zheng T, Lan Q, Zhang Y, Zahm SH, Wang SS, Holford TR, Leaderer B, Yeager M, Welch R, Kang D, Boyle P, Zhang B, Zou K, Zhu Y, Chanock S, Rothman N (2006) Polymorphisms in DNA repair genes and risk of non-Hodgkin lymphoma among women in Connecticut. Hum Genet 119:659–668
9. Hill DA, Wang SS, Cerhan JR, Davis S, Cozen W, Severson RK, Hartge P, Wacholder S, Yeager M, Chanock SJ, Rothman N (2006) Risk of non-Hodgkin lymphoma (NHL) in relation

to germline variation in DNA repair and related genes. Blood 108:3161–3167
10. Wang Z, Xu Y, Tang J, Ma H, Qin J, Lu C, Wang X, Hu Z, Wang X, Shen H (2009) A polymorphism in Werner syndrome gene is associated with breast cancer susceptibility in Chinese women. Breast Cancer Res Treat 118:169–175
11. Wirtenberger M, Frank B, Hemminki K, Klaes R, Schmutzler RK, Wappenschmidt B, Meindl A, Kiechle M, Arnold N, Weber BH, Niederacher D, Bartram CR, Burwinkel B (2006) Interaction of Werner and Bloom syndrome genes with p53 in familial breast cancer. Carcinogenesis 27:1655–1660
12. Yoshida T, Kato K, Yokoi K, Watanabe S, Metoki N, Satoh K, Aoyagi Y, Nishigaki Y, Nozawa Y, Yamada Y (2009) Association of candidate gene polymorphisms with chronic kidney disease in Japanese individuals with hypertension. Hypertens Res 32:411–418
13. Ye L, Miki T, Nakura J, Oshima J, Kamino K, Rakugi H, Ikegami H, Higaki J, Edland SD, Martin GM, Ogihara T (1997) Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. Am J Med Genet 68:494–498
14. Morita H, Kurihara H, Sugiyama T, Hamada C, Yazaki Y (1999) A polymorphic variant C1367R of the Werner helicase gene and atherosclerotic diseases in the Japanese population. Thromb Haemost 82:160–161
15. Nakayama R, Sato Y, Masutani M, Ogino H, Nakatani F, Chuman H, Beppu Y, Morioka H, Yabe H, Hirose H, Sugimura H, Sakamoto H, Ohta T, Toyama Y, Yoshida T, Kawai A (2008) Association of a missense single nucleotide polymorphism, Cys1367Arg of the WRN gene, with the risk of bone and soft tissue sarcomas in Japan. Cancer Sci 99:333–339
16. Lan Q, Zhang L, Shen M, Jo WJ, Vermeulen R, Li G, Vulpe C, Lim S, Ren X, Rappaport SM, Berndt SI, Yeager M, Yuenger J, Hayes RB, Linet M, Yin S, Chanock S, Smith MT, Rothman N (2009) Large-scale evaluation of candidate genes identifies associations between DNA repair and genomic maintenance and development of benzene hematotoxicity. Carcinogenesis 30:50–58
17. Ogata N, Shiraki M, Hosoi T, Koshizuka Y, Nakamura K, Kawaguchi H (2001) A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J Bone Miner Metab 19:296–301
18. Orimo H, Hayashi Y, Fukunaga M, Sone T, Fujiwara M, Shiraki M, Kushida K, Miyamoto S, Soen S, Nishimura J, Oh-hashi Y, Hosoi T, Gorai I, Tanaka H, Igai T, Kishimoto H (2001) Diagnostic criteria for primary osteoporosis: year 2000 revision. J Bone Miner Metab 19:331–337
19. Grove ML, Yu B, Cochran BJ, Haritunians T, Bis JC, Taylor KD, Hansen M, Borecki IB, Cupples LA, Fornage M, Gudnason V, Harris TB, Kathiresan S, Kraaij R, Launer LJ, Levy D, Liu Y, Mosley T, Peloso GM, Psaty BM, Rich SS, Rivadeneira F, Siscovick DS, Smith AV, Uitterlinden A, van Duijn CM, Wilson JG, O'Donnell CJ, Rotter JI, Boerwinkle E (2013) Best practices and joint calling of the HumanExome BeadChip: the CHARGE consortium. PLoS One 8:e68095
20. Fuku N, Mori S, Murakami H, Gando Y, Zhou H, Ito H, Tanaka M, Miyachi M (2012) Association of 29C > T polymorphism in the transforming growth factor-β1 gene with lean body mass in community-dwelling Japanese population. Geriatric Gerontol Int 12:292–297
21. Mori S, Fuku N, Chiba Y, Tokimura F, Hosoi T, Kimbara Y, Tamura Y, Araki A, Tanaka M, Ito H (2010) Cooperative effect of serum 25-hydroxyvitamin D concentration and a polymorphism of transforming growth factor-beta1 gene on the prevalence of vertebral fractures in postmenopausal osteoporosis. J Bone Miner Metab 28:446–450
22. Solé X, Guinó E, Valls J, Iniesta R, Moreno V (2006) SNPStats: a web tool for the analysis of association studies. Bioinformatics 22:1928–1929
23. Jacobson HG, Rifkin H, Zucker-Franklin D (1960) Werner's syndrome: a clinical-roentgen entity. Radiology 74:373–385
24. Murata K, Nakashima H (1982) Werner's syndrome: 24 cases reviewed in the Japanese literature. J Am Geriatr Soc 30:303–308
25. Walton NP, Brammar TJ, Coleman NP (2000) The musculoskeletal manifestations of Werner's syndrome. J Bone Joint Surg Br 82:885–888
26. Shiraki M, Aoki C, Goto M (1988) Bone and calcium metabolism in Werner's syndrome. Endocr J 45:505–512
27. Rubin CD, Zerwekh JE, Reed-Gitomer BY, Pak CY (1992) Characterization of osteoporosis in a patient with Werner's syndrome. J Am Geriatr Soc 40:1161–1163
28. Laroche M, Ricq G, Cantagrel A, Amigues JM, Mazieres B (1997) Bone and joint involvement in adults with Werner's syndrome. Rev Rhum Engl Ed 64:843–846
29. Pignolo RJ, Suda RK, McMillan EA, Shen J, Lee SH, Choi Y, Wright AC, Johnson FB (2008) Defects in telomere maintenance molecules impair osteoblast differentiation and promote osteoporosis. Aging Cell 7:23–31
30. Wang H, Chen Q, Lee SH, Choi Y, Johnson FB, Pignolo RJ (2012) Impairment of osteoblast differentiation due to proliferation-independent telomere dysfunction in mouse models of accelerated aging. Aging Cell 11:704–713
31. Matsumoto T, Imamura O, Yamabe Y, Kuromitsu J, Tokutake Y, Shimamoto A, Suzuki N, Satoh M, Kitao S, Ichikawa K, Kataoka H, Sugawara K, Thomas W, Mason B, Tsuchihashi Z, Drayna D, Sugawara M, Sugimoto M, Furuichi Y, Goto M (1997) Mutation and haplotype analyses of the Werner's syndrome gene based on its genomic structure: genetic epidemiology in the Japanese population. Hum Genet 100:123–130
32. Tadokoro T, Rybanska-Spaeder I, Kulikowicz T, Dawut L, Oshima J, Croteau DL, Bohr VA (2013) Functional deficit associated with a missense Werner syndrome mutation. DNA Repair 12:414–421
33. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL et al (2012) Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 44:491–501