Spectrum and risk of neoplasia in Werner syndrome

Julia Lauper

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington

2012

Committee:
Thomas L. Vaughan
Raymond J. Monnat, Jr.

Program Authorized to Offer Degree:
Epidemiology – Public Health

University of Washington

Abstract

Spectrum and risk of neoplasia in Werner Syndrome

Julia Lauper

Chair of the Supervisory Committee:
Professor Thomas L. Vaughan
Epidemiology

Background: Werner syndrome (WS) is an autosomal recessive genetic instability and progeroid syndrome that is associated with an elevated risk of cancer.

Methods: We used case reports of neoplasms in WS patients identified in previous case series or newly identified through searches of PubMed, Google Scholar and J-EAST (a database of articles from Japan) to define the spectrum (types and sites) of neoplasia in WS. Neoplasm type-specific risk was calculated by determining standardized incidence and proportionate incidence ratios (SIR and SPIR, respectively) of neoplasms in Japan-resident WS patients versus population control data from the Osaka prefecture and Osaka Cancer Registry.

Results: Our study population consisted of 188 WS patients with 246 neoplasms. The most frequent neoplasms in WS patients, representing 2/3 of all reports, were thyroid neoplasms, malignant melanoma, meningioma, soft tissue sarcomas, leukemia and pre-leukemic conditions of the bone marrow, and primary bone neoplasms. SIRs were significantly elevated in Japanese WS patients for the five most frequent cancer types and meningiomas, from 83.2-fold for melanomas of the skin (95% CI: 45.5, 139.6) to 3.6-fold for leukemias (95% CI: 1.7, 6.9), and for all five cancer types except leukemias by SPIR analysis.

Conclusions: WS confers a strong predisposition to several specific types of neoplasia, and this spectrum differs between WS patients residing in, as opposed to outside, Japan. These results provide a guide for WS clinical care and cancer screening, and for analyses to define the mechanistic basis for cancer in WS and in the general population.

TABLE OF CONTENTS

Acknowledgements

Thank you to my advisors, Tom and Ray, for providing wisdom, patience, and good humor in mentoring me on this project. Thank you to Alison, whose translation skills and friendship were invaluable. Thank you to the members of the Monnat lab for providing feedback, translation assistance, and guidance on this project. Lastly, thank you to my family and to Jack for their support.

This work was supported by an Interdisciplinary Training Grant in Cancer Research pre-doctoral training award from the National Institutes of Health (T32CA080416 to J.L.); a scholarship from the Northern California Scholarship Foundation to J.L.; a career award from the National Cancer Institute at the National Institutes of Health (K05CA124911 to Dr. Vaughan); and by a P01 Program award from the National Cancer Institute at the National Institutes of Health (CA77852 to Dr. Monnat).

We thank Dr. Hideaki Tsukuma for help with Osaka Cancer Registry data and Osaka population statistics; Dr. Hiroshi Mori for providing additional information on a tumor in a WS patient; and Drs. Ellen Wijsman and Mary Emond for advice on determining the prevalence of WS in Japan. We had help in case finding from Helen Tang, Alden Hackmann and the University of Washington Interlibrary Loan Service. Dr. Stefan Pellenz and Melissa Lucas assisted with translations and data collection. Kazumi Tsukazawa, Hollis Crapo, and Dr. Hui Li also assisted with translation. Statistical software was provided through the Center for Studies in Demography and Ecology (CSDE) and the Student Technology Fee at the University of Washington.

Introduction

Werner syndrome (WS, OMIM #277700) is an autosomal recessive disease caused by loss of function mutations in the *WRN* gene. WS patients develop features reminiscent of premature aging beginning in the second decade of life. These progeroid features include bilateral cataracts, graying and loss of hair, scleroderma-like skin changes, diabetes mellitus, and osteoporosis (1). WS patients are also at elevated risk for common, clinically important age-dependent diseases such as atherosclerotic cardiovascular disease and associated sequelae (myocardial infarction, stroke and peripheral vascular disease) and cancer. These last two disease processes–cardiovascular disease and cancer–are collectively responsible for ~85% of deaths in molecularly-confirmed WS patients at a median age of 54 years (1-3).

The elevated risk of cancer in WS patients was noted early, and emphasized as a cardinal feature of WS (1). Many of the features of cancer arising in WS patients support the idea that WS is a cancer predisposition syndrome. These features include early age of onset compared with the general population; an elevated risk of specific tumor types, including uncommon tumor types; unusual tumor sites; and the presence of multiple, often histologically distinct tumors in individual patients (4). The most frequent tumor types reported in WS include soft tissue sarcomas, meningiomas, melanomas that arise in non-sun-exposed sites and mucosal surfaces, thyroid neoplasms, and malignant and pre-malignant hematologic abnormalities (1, 4-6).

In order to better define the spectrum (types and sites) and quantify the increased risk of neoplasia in WS, we performed comprehensive searches to identify case reports of neoplasms in WS patients worldwide. This newly assembled study population was used first to define the spectrum of neoplasia in WS patients. We then used the largest subset, of WS patients residing in Japan, to estimate the type-specific risk of neoplasia in WS patients relative to population control data from the Osaka prefecture and Osaka Cancer Registry. Our results provide a detailed and quantitatively rigorous view of cancer types and risk in WS. These results will guide

the clinical care of WS patients, and efforts to understand the mechanistic origins of cancer in WS and in the general population.

Methods

WS case-finding: Case reports of neoplasms arising in WS patients were identified by reviewing all available, previously published case reports or series (see, e.g.,(5)), and by new searches to identify additional case reports in Japanese, English or other languages. Case reports of WS individuals with neoplasia from Japan were identified by searching J-EAST (http://sciencelinks.jp/j-east/) using the search terms <“Werner” OR “Werner’s” AND “syndrome”>. Additional case reports of individuals with neoplasia from Japan were identified by searching PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using the following search terms:

<"werner syndrome"[MeSH Terms] OR ("werner"[All Fields] AND "syndrome"[All Fields]) OR "werner syndrome"[All Fields] OR ("werner's"[All Fields] AND "syndrome"[All Fields]) OR "werner's syndrome"[All Fields]) AND ("case reports"[Publication Type] OR "case report"[All Fields]).

To identify cases of WS individuals with neoplasia outside of Japan, we conducted searches in PubMed and Google Scholar (http://scholar.google.com/) using the search terms <‘Werner’>, <‘Werner’s’> and <‘Syndrome’> and <‘Werner’s syndrome case report’> in conjunction with <‘cancer’>, <‘tumor’> and <‘neoplasm’>. Articles were reviewed in full if they referred to WS and noted a tumor or neoplasm in the title or abstract. Some additional reports of neoplasia in WS were identified by reviewing WS case reports that did not specifically refer to a ‘tumor,’ ‘neoplasm’ or ‘cancer’ in the title or abstract. Searches were complete by 1 September 2011.

The diagnosis of Werner syndrome was independently assessed using case data and WS diagnostic criteria established by the International Registry of Werner Syndrome (http://www.wernersyndrome.org/registry/diagnostic.html). We classified the diagnosis of WS as ‘definite’, ‘probable’, ‘possible’ or ‘uncertain’ for subsequent sensitivity analyses (Supplementary Table 1). We also collected patient gender, cause of death and age at death (where specified), and neoplasm-specific data including year of tumor diagnosis (when unspecified, the year prior

to the year of publication was substituted), age at tumor diagnosis, pathologic diagnosis, a diagnosis of malignancy, anatomic site(s), whether a finding was incidental at autopsy, neoplasm gross and histopathology descriptions, and whether images of neoplasm gross morphology or histopathology were provided. Reported tumor diagnoses were verified by comparing the stated diagnosis with patient-specific gross pathology and histopathology images whenever available.

Recurrent and multiple neoplasms of the same type at the same site were not counted as independent primary tumors, following previously established guidelines for reporting multiple primary neoplasms (7). We used a combination of tumor and patient data to exclude multiple reporting of patients or tumors in our final study population (reviewed by J.M.L., A.K., and R.J.M, Jr.). Data from multiple reports of the same patient were pooled whenever possible to improve data completeness (see cases with multiple references in Supplementary Spreadsheet 1). We excluded tumor reports from our study population which: reported an unspecified malignancy (could not be assigned to ICD-10 C00-96but44); lacked patient-specific clinical or demographic data; could not be traced to a primary case report describing the patient or the patient's relation; or potentially duplicated reports already included in the study population. These tumor reports were separately cataloged for reference. Personal observations or communications of tumors reported by authors of published articles were considered primary case reports.

Population comparison data: Comparison population-based cancer incidence data were obtained from the WHO *Cancer Incidence in Five Continents* (*CI5*) publication data available online (8). Japanese population comparison data for relative risk estimation was from the Osaka prefecture, which has both a large population and a long-established cancer registry. In order to quantify the relative risk of benign meningiomas which are not covered in WHO *CI5* data, we used additional data provided by the Osaka Cancer Registry. These data included de-identified individual case records of benign and malignant meningiomas (ICD-10 codes C70.0-70.9, D32.0-32.9, D42.0-42.9) categorized by gender, age and year at diagnosis. Similar case data

on malignant cutaneous melanoma (ICD-10 code C43.0-43.9) were used to assess the relative risk for acral lentiginous melanoma (ICD-O-3M code 8744/3).

Case reports and population comparison data were publicly available and/or de-identified. Thus our data collection and analysis plan could be performed without IRB approval as assessed by the University of Washington Human Subjects Division (reference #42092) and the Ethical Committee of Osaka Medical Center for Cancer and Cardiovascular Diseases (request #11-0006 for Osaka Cancer Registry data).

Spectrum of neoplasia analyses: Neoplasms were classified by histopathologic type and site using case report data and diagnoses, with reinterpretation or translation where needed to match current diagnostic classification criteria and WHO International Classification of Diseases for Oncology (ICD-O) nomenclature and guidelines (7). When there was ambiguity as to neoplasm histopathologic type, cancers or malignancies not otherwise specified were assumed to be carcinomas at the following organ sites: breast, larynx, esophagus, ovary, thyroid, uterus, nasal cavity and liver. Unspecified stomach and pancreas neoplasms were assumed to be adenocarcinomas. The most frequent neoplasms by type and site were identified using all study population case data, and then subdivided into neoplasms arising in Japan-resident patients or in patients residing outside of Japan regardless of ethnicity.

Histopathologic and geographic spectrum analyses: We compared the frequency of thyroid malignancy subtypes in Japan-resident WS patients to Osaka prefecture comparison data *CI5* (*CI5* volumes 7-9, years 1988-2002, available from http://ci5.iarc.fr/CI5i-ix/ci5i-ix.htm) (9-11) using Fisher's exact test. Thyroid malignancies were classified into one of the following subtypes for this analysis: papillary, follicular, other or unspecified. We also tested whether the frequency of several neoplasms differed significantly between Japan-resident patients and those residing outside of Japan. The neoplasms analyzed in this way were soft tissue sarcomas, bone neoplasms, malignant melanomas, meningiomas, hematologic/lymphoid neoplasms, thyroid neoplasms, or all other neoplasms. A global Fisher's exact test was performed to detect

overall differences in the distribution of these neoplasms between patient subgroups. Local Fisher's tests were also conducted for each neoplasm type or group.

Relative risk analyses: We calculated neoplasm-specific standardized proportionate incidence ratios (SPIRs) to determine the extent to which the most frequently reported neoplasms were over-represented in Japan-resident WS patients versus the reference population. The proportions of specific malignant neoplasms observed in WS patients between 1965-2009 were compared to the proportions expected based on *CI5* data for the Osaka prefecture between 1970-2002, adjusted for age (10-39y and 40-69y) and time period (prior to 1988, and ≥1988). SPIRs were calculated for soft tissue and connective tissue malignancies (ICD-10 C47 & C49); bone malignancies (ICD-10 C40-41); malignant melanomas of the skin (ICD-10 C43); leukemias (ICD-10 C91-95); and thyroid malignancies (ICD-10 C73). For comparison we estimated the SPIR for GI malignancies (ICD-10 codes C15-25), where we expected little or no elevated risk in WS patients based on prior data (4-6). Each proportion was calculated as the fraction of all malignancies observed, except for non-melanoma skin cancers (ICD-10 C00-96 but C44). We excluded the following neoplasms reported in WS patients from this analysis: non-malignant tumors (except meningiomas, which are included in 'ICD-10 C00-96 but C44' counts prior to 1988); incidental findings at autopsy; non-melanoma skin cancers (ICD-10 C44); tumors reported without an age at tumor diagnosis (to prevent multiple counting of the same tumor); and tumors diagnosed after 2009. Proportions were compared using indirect standardization (12).

Standardized incidence ratios (SIRs) were also calculated to estimate neoplasm-specific relative risk for the malignant neoplasms assessed by SPIR, as well as for all malignancies except non-melanoma skin cancers (ICD-10 C00-96 but C44) and meningiomas (ICD-10 codes C70.0-70.9, D32.0-32.9, D42.0-42.9). We estimated the SIR by comparing the observed number of cases in WS to the expected number of cases calculated from gender- and age-specific population incidence rates and the estimated number of WS patients in Japan. We

calculated annual estimates of the number of WS patients in Japan from the frequency estimates of pathogenic alleles in the Japanese population (q=0.0014 to q=0.006)(13,14), and the total population size by using the Hardy-Weinberg equilibrium: $n_{WS} = n_{pop}*q^2$, where

q = the allele frequency of WS pathogenic alleles in the population

n_{WS} = number of WS patients

n_{pop} = number of individuals in the population

For initial estimates we used the average of pathogenic allele frequency estimates (i.e., q=0.0037), and n_{pop}, data from the 1965-2009 annual Japan population estimates from the Statistics Bureau of Japan (Table 2-3 at http://www.stat.go.jp/english/data/chouki/02.htm).

In order to estimate the age distribution of WS patients, we used Japanese population data that included population estimates by gender and 5-year age grouping between 1965 and 2009 (see above). We adjusted the age distribution of the general population to account for the shortened life expectancy in WS, where the median age at death is 54.3 years (2, 15), then estimated the WS patient population at risk by age interval. We assumed the proportion of the WS population age 0-29 yrs would be the same as the proportion of the general Japan population age 0-29 years. Thereafter, we assumed each 15-year age grouping in the general population up to age 74 yrs was equivalent to a 10-year age group in WS (e.g., general population ages 30-44 yrs was equivalent to WS patient ages 30-39 yrs). At the upper age range, the proportion of individuals ages 75+ yrs in the general population was considered equivalent to the proportion of WS patients at ages 60+ yrs. This method accounts for trends in Japanese birth and death rates over time. For sensitivity analyses, we generated alternate WS patient population distributions by changing the earliest age at which we assume the general population age distribution differs from the WS population age distribution (i.e., starting at age 20 yrs or at 40 yrs).

Osaka prefecture neoplasm-specific population incidence rates were obtained from CI5 data in which annual cases were categorized by gender and divided into 5-year age groups from

1963-2002. The CI5*plus* online analysis application (http://ci5.iarc.fr/CI5plus/ci5plus.htm, see (8)) was used to obtain case numbers for several malignancies, including: bone malignancies (ICD-10 C40-41), malignant melanomas of the skin (ICD-10 C43), leukemias (ICD-10 C91-95), thyroid malignancies (ICD-10 C73), and all sites but non-melanoma skin (ICD-10 C00-96 excluding C44). Because the CI5*plus* online analysis application does not provide annual case numbers for malignancies of the soft and connective tissue (ICD-10 C47 & C49) or for all GI malignancies (ICD-10 C15-25), we obtained these data from *CI5* volumes. Additionally, cases of meningeal neoplasms (ICD-10 codes C70.0-70.9, D32.0-32.9, D42.0-42.9) categorized by gender and divided into 5-year age groupings were obtained from data provided by the Osaka Cancer Registry. Incidence rates as a function of time period were calculated using these case numbers and annual Osaka population estimates categorized by gender and divided into 5-year age groups as provided by the Osaka Cancer Registry. Available incidence rates were then applied to any years with missing data (e.g., reported incidence rates from 1970-1971 were applied to 1965-1969 to obtain the likely expected case numbers within that time period).

The SIR was calculated as the ratio of the total number of WS tumor cases of a specific tumor type observed from 1965-2009 to the expected number. In determining the number of observed WS cases by tumor type, the exclusion criteria used for SPIR analyses were followed for SIR, with the exception that all meningiomas were included in SIR estimates for meningioma. The expected number of cases was calculated using indirect standardization by applying the population incidence rates to the estimated WS patient population, with rates adjusted for gender, 10-year age groupings from ages 10-69 (population incidence rates for ages 60-69 were applied to WS patients estimated to be ages 60+) and time period (2-6 year intervals based on periods covered by *CI5* volumes from 1965-2009). Exact 95% confidence intervals were obtained for each estimate. For sensitivity analyses, we re-calculated the WS patient population at risk based on the upper (q=0.006)(14) and lower (q=0.0014)(13) allele frequency

estimates. Sensitivity analyses were conducted for both the SIR and SPIR analyses using Japan-resident WS cases with ‘definite’ or ‘probable’ WS diagnoses.

Statistical methods: All analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA). Fisher’s exact tests were two-sided. For the neoplasm-specific tests of geographic variation in tumor type, a Bonferroni correction was applied to the $\alpha = 0.05$ threshold for statistical significance. In both SPIR and SIR analyses the exact 95% confidence interval for each estimate was obtained using the “istdize” command in STATA.

Results

We identified 188 Werner syndrome (WS) patients with 246 neoplasms reported between 1939 and 2011. This population included 138 Japan-resident patients, and an additional 50 patients from a diversity of locations and ethnicities outside of Japan (Table 1). Among the 188 patients in our study population, the mean age at first diagnosis of neoplasia was 43.3 years $\pm$ 9.9 years (range 20 - 69). The study population is summarized in greater detail in Supplementary Spreadsheet 1. We excluded additional tumor reports from our study population based on specified exclusion criteria (n=87; Supplementary Spreadsheet 2: Additional case reports not included in the study population)

Spectrum of neoplasia in WS: The most frequent neoplasms in WS patients, representing 67% of all reports, were thyroid neoplasms, malignant melanoma, meningioma, soft tissue sarcomas, leukemia and pre-leukemic conditions of the blood and bone marrow, and osteosarcoma/bone neoplasms (Table 2). Malignant melanomas were almost exclusively less common variants: acral lentiginous melanomas arising on the palms and soles, or mucosal melanomas arising in the nasal cavity or esophagus. Thyroid neoplasms in Japan-resident patients included a disproportionately high number of follicular carcinomas, in contrast to the more common papillary variant observed in the general population ($p=0.00002$, using reference population ages 0-24, and $p=0.00001$ for reference population ages 10-69; see Supplementary Table 2). In contrast, thyroid neoplasms were less frequent in WS patients residing outside Japan, though the difference was not statistically significant after correcting for multiple testing ($p=0.016$). The frequency of melanomas was lower, though not significant reduced, among WS patients residing outside Japan ($p=0.082$) (Table 3). Leukemias covered the full spectrum of morphologies, though we lacked data to assign a consistent WHO and/or FAB classification to all case reports. Atypical leukemias and a wide range of preleukemic disorders were also reported, including myelodysplasia, myelofibrosis and refractory anemia with an excess of

blasts (RAEB) (Table 2). Multiple neoplasms were common: 22% of WS patients (41/188) had 1 to 4 additional concurrent or sequential neoplasms. These were often at different sites, and of substantially different histopathologic types (Table 4).

Standardized proportional incidence ratios (SPIRs) for malignancy in WS: We estimated the elevated risk of malignant neoplasms in WS by calculating SPIRs using data from the Japan-resident cases and comparison data from the Osaka Japan prefecture (Table 5). These analyses revealed strongly elevated SPIRs for melanomas of the skin (SPIR=56.1, 95% CI: 30.7, 94.2), soft tissue malignancies (SPIR=20.7, 95% CI: 11.6, 34.1), and bone malignancies (SPIR=11.6, 95% CI: 5.8, 20.8). The proportionate incidence of thyroid malignancy was elevated, though to a lesser degree (SPIR=9.1, 95% CI: 5.7, 13.8), and there was no statistically elevated risk for leukemia excluding preleukemic disorders (SPIR=1.7, 95% CI: 0.78, 3.3). We observed a decrease in the proportion of GI malignancies in WS patients versus the comparison population (SPIR=0.23, 95% CI: 0.13, 0.37). This decrease is expected: in SPIR analyses a substantial elevation in incidence of one group of cancers will necessarily reduce the SPIR for cancers not related to WS, since the proportions must sum to 100%. This also may reflect a real decrease in risk or the under-reporting of common neoplasms such as GI malignancies in WS or similar cancer predisposition syndromes, where case reporting has historically focused on less frequent types or sites of neoplasia.

Sensitivity analyses were performed to determine how SPIR estimates were modified as a function of confidence in the diagnosis of WS (Supplementary Table 3). When we included only Japan-resident WS cases with either ‘definite’ or ‘probable’ diagnoses of WS in SPIR estimates, we continued to observe significant elevations in the proportionate incidence of all of the common neoplasms in WS except leukemia. The highest risk observed in this analysis was for melanomas of the skin (SPIR=65.2, 95% CI: 31.3, 120.0).

Standardized incidence ratios (SIRs) for neoplasia in WS: We calculated SIRs as a complementary method for quantifying the risk of neoplasia in WS patients. SPIRs provide a

measure of the over-representation of a particular disease type in a cohort when incident cases are collected, but the size of the underlying population at risk is not known (12). However, the SPIR estimates may be biased if case ascertainment is biased. For example, cancer types that are rare in the general population are more likely to be reported in the clinical literature when they are identified in WS patients. In contrast, SIR estimates are not affected by under-reporting of other cancers, but do require estimating the population size, age and gender distribution of individuals at risk. SIR analyses with the WS population at risk, estimated using the average of reported WS pathogenic allele frequencies (q=0.0037)(13), indicated significantly elevated risk for all six common tumor types observed in Japan-resident WS patients. These SIR risk estimates ranged from 83.2 (95% CI: 45.5, 139.6) for melanomas of the skin to 3.6 (95% CI: 1.7, 6.9) for leukemias (see Table 6). We could not further quantify the risk of acral lentiginous melanoma in WS, as there were too few cases in Osaka Cancer Registry data to provide a comparison population (n=5, from 1963-2005). A decreased risk of GI malignancy in WS (SIR=0.31, 95% CI: 0.18, 0.50), was less than that observed in SPIR analyses (see above). The overall risk of malignancy of all types in WS patients was 30% higher than in an Osaka prefecture reference population (SIR=1.3, 95% CI: 1.1, 1.6).

SIR sensitivity analyses were performed to determine the influence of pathogenic *WRN* allele frequency estimates on risk estimates (Supplementary Table 4). Neoplasm type-specific SIRs calculated using the highest estimated pathogenic *WRN* allele frequency (q=0.006)(14) were 2.5-fold lower than estimates using an average allele frequency (see above), but still significantly increased for melanomas of the skin (SIR=31.7, 95% CI: 17.3, 53.1), benign or malignant meningiomas (SIR=20.6, 95%: 11.5, 34.0), soft tissue malignancies (SIR=16.2, 95% CI: 9.0, 26.6), bone malignancies (SIR=12.6, 95% CI: 6.3, 22.5) and thyroid malignancies (SIR=5.3, 95% CI: 3.3, 8.1) (Supplementary Table 4). However, we no longer detected a statistically significant increase in the risk of leukemia associated with WS (SIR=1.4, 95% CI: 0.63, 2.6), and observed an even lower estimated risk of GI malignancies (SIR=0.12, 95% CI:

0.07, 0.19) and malignancies of all types (SIR=0.51, 95% CI: 0.42, 0.60). The same analysis performed using the lowest estimated *WRN* pathogenic allele frequency (q=0.0014)(13) indicated a 7-fold increase in risk across malignancies of all types compared to the estimates using an average allele frequency (see above), and a 2-fold higher risk of GI malignancies in WS patients compared with the Osaka reference population (SIR=2.2, 95% CI: 1.2, 3.5). These altered risk estimates directly reflect the different estimated populations of WS patients at risk of neoplasia.

Sensitivity analyses were also conducted to determine the influence of the age distribution and WS diagnostic confidence on risk. These analyses provided similar relative risk estimates to those obtained in the main analysis when conditioned on age distribution (Supplementary Table 5), and a significantly elevated risk of neoplasia for all common neoplasm types observed in WS except leukemia when conditioned on diagnostic confidence (Supplementary Table 6). The elevated relative risk estimates for the highest diagnostic confidence cases ranged from 59.4-fold for melanomas of the skin (95% CI: 28.5, 109.3) to 8.9-fold for thyroid malignances (95% CI: 4.9, 15.0).

Discussion

Our goal in this study was to provide a comprehensive and quantitatively rigorous analysis of the spectrum and excess risk of specific neoplasms in WS, a heritable cancer predisposition and progeroid syndrome. We reasoned that the results of our analyses would guide the care of contemporary WS patients, while providing clues to the mechanistic basis for cancer in WS. Our analyses were performed with a study population of 188 WS patients having 246 different neoplasms that was assembled from previous case reports and unpublished case material. A majority of our study population consisted of WS patients residing in Japan (n=138, or 73% of patients). Additional patients were from a wide diversity of locations outside Japan. The large Japan-resident portion of cases allowed us to calculate neoplasm-specific, as well as overall relative risk of neoplasia as Japanese population reference data could be used for comparisons.

Two-thirds (67%) of the neoplasms reported in our study population patients were of six major histopathologic types (Table 2). In order of decreasing frequency these were: thyroid neoplasms; malignant melanomas; meningiomas; soft tissue sarcomas; leukemias and preleukemic disorders such as myelodysplasia; and osteosarcomas/bone neoplasms. We again observed differences in the frequency of thyroid neoplasms, and to a lesser degree melanomas, between Japan-resident WS patients and WS patients residing elsewhere. However these differences were not statistically significant (Table 3) (5). The geographic difference in frequency of these two neoplasms in WS cannot be explained by differences in the frequency of different *WRN* mutations: many of the same mutations have been found in both populations, and all clinically ascertained *WRN* mutations share a common null biochemical phenotype (16-18). Both thyroid follicular carcinoma and acral lentiginous melanoma have distinctive molecular and genetic features (19-25). These features may be useful in dissecting geographic contributions to risk, and in identifying new genetic or environmental modifiers to neoplasm-specific risk in WS.

The strong, heritable cancer predisposition in WS is reflected in the multiple instances of uncommon neoplasms, and the high frequency of multiple neoplasms (41/188 patients, or 22%; Table 4) in our WS study population. The spectrum of neoplasia in WS overlaps with, but is distinct from, the spectrum of neoplasms observed in two other RECQ helicase deficiency syndromes, Bloom syndrome and Rothmund-Thomson syndrome. Bloom syndrome (BS) is a very strong predisposition to a wide range of types of neoplasia (26, 27), while in Rothmund-Thomson syndrome (RTS) the cancer predisposition is largely limited to osteosarcoma (28, 29). The spectrum of neoplasms observed in WS also overlaps in part with other recessive cancer predisposition syndromes such as Li-Fraumeni syndrome (30, 31); other heritable marrow failure syndromes such as Fanconi anemia (32, 33), dyskeratosis congenita (34), Diamond-Blackfan anemia (35) or Shwachman-Diamond syndrome (36, 37); and mismatch repair deficiency syndromes (e.g., *MLH1* or *PMS2*) (38).

Our analysis provides a comprehensive analysis of neoplasm type-specific excess risk in WS using population-based cancer registry data and the complementary analytic approaches of SPIR and SIR estimation. These analyses (Tables 5 and 6) confirmed the substantially elevated risk of developing the most frequent neoplasms observed in WS, with the exception of leukemia where risk was statistically significantly elevated in only a portion of analyses. However, we suspect that the risk of leukemia is significantly elevated in WS, as many leukemia cases have been reported together with many reports of preleukemic disorders such myelodysplasia (Table 2). Moreover, we and others have reported age-dependent increase in genetic instability in peripheral blood in WS patients (39-41). Sensitivity analyses conditioned on WS diagnostic confidence, mutant allele frequency or WS patient age distribution all showed our risk estimates to be robust. The low risk of GI malignancies in WS relative to the general population (see Tables 5 and 6) may reflect reduced risk in WS patients, or more likely the under-reporting of common malignancies in WS patients.

There are several potential limitations and sources of uncertainty in our analysis. Because we relied on case reports to assemble our study population there were ambiguities in some instances in tumor diagnosis (e.g., in distinguishing thyroid goiter from adenomas among Japan residents), or in clearly assigning an age at tumor diagnosis. We resolved these and other uncertainties using available data and common decision criteria wherever possible (see Supplementary Spreadsheet 2). WS is under-diagnosed and under-reported everywhere including in Japan, by virtue of an incompletely penetrant phenotype with delayed onset (1, 2, 42). However, case underreporting is unlikely to strongly bias our risk estimates as underreporting biases SIR estimates toward the null hypothesis (i.e., no increase in risk). Lastly, we used sensitivity analyses to assess the influence of WS diagnostic confidence and the estimated WS population on risk estimates. These analyses revealed quantitative differences, though none that altered our overall finding of elevated risk of specific types of neoplasia. It should be possible to further refine these estimates in the future using emerging population-based analyses of *WRN* mutant allele frequencies in Japan or other populations.

Despite the potential limitations of our case-based analyses we think our risk estimates are both conservative and robust. This analysis clearly identify specific types and sites of neoplasia that occur with high frequency in WS patients, and at rates substantially higher than in the general population. All of these sites of common neoplasms with the exception of bone marrow are conditionally dividing cell types or lineages where genetic instability and cell turnover may lead to the emergence of neoplastic clones. These cell lineages are also notable for the lack of strong, non-specific tumor-suppressive mechanisms such as damage- or developmentally-induced apoptosis, terminal differentiation or senescence (18). While treatment of a first primary neoplasm could contribute to the elevated risk of neoplasia in some WS patients, there has not been a well-documented instance of a therapy-induced neoplasm in WS. There are, however, reports of heightened sensitivity to chemotherapy leading to unintended, therapy-related toxicity or death (43).

While our analysis has focused on the risk of neoplasia in WS patients, *WRN* mutations may also be contributing to the population burden of cancer. The largest likely contribution reflects the high frequency of deleterious germline *WRN* mutations in virtually all populations. For example, even conservative *WRN* mutant allele frequency estimates predict greater than 1 million heterozygous carriers of pathogenic *WRN* mutations in the U.S. Heterozygous carriers display low levels of genetic instability *in vivo* (41), and at the cellular level display intermediate sensitivity to cell killing by DNA damaging agents (44). This combination of genetic instability and DNA damage sensitivity could further predispose to the mutation or loss of the remaining wildtype *WRN* allele, or other genes that influence cancer risk or progression in the presence of *WRN* haploinsufficiency. Genomic and population-based analyses of cancer in *WRN* mutation carriers and in sporadic cancers of the types most frequently observed in WS should provide additional insight into the origins of cancer in WS, and the contribution of *WRN* mutations to cancer risk in the general population.

Tables

Table 1: Werner syndrome neoplasia study population

WS patient type	patients (n)	gender (M/F/ns*)	mean age at first neoplasm diagnosis (in yrs)**	age at first neoplasm diagnosis (range, in yrs.)**	neoplasms (n)	number/percentage of multiple neoplasm patients
residing in Japan	138	79/57/2	44.1	20-69	182	31 (22%)
residing outside Japan	50	23/26/1	41.4	23-56	64	10 (20%)
Total	**188**	**102/83/3**	**43.3**	**20-69**	**246**	**41 (22%)**

* ns = not specified or reported

** among patients whose age at diagnosis is specified.

Table 2: Spectrum of neoplasia in Werner syndrome patients*

Frequent neoplasms (67% of total, n = 164)	**Less common neoplasms** (33% of total, n = 82)
thyroid neoplasms (15.9%, n = 39) follicular thyroid carcinoma papillary thyroid carcinoma anaplastic thyroid carcinoma thyroid adenoma **malignant melanoma** (13.0%, n = 32) acral lentiginous melanoma (ALM) malignant mucosal melanoma malignant melanoma non-ALM **meningioma** (11.0%, n = 27) **soft tissue sarcomas** (9.8%, n = 24) undifferentiated pleomorphic sarcoma (malignant fibrous histiocytoma) leiomyosarcoma fibrosarcoma malignant peripheral nerve sheath tumor rhabdomyosarcoma synovial sarcoma **hematologic/lympho**id (9.3%, n = 23) acute myelogenous leukemia (M1-M5, M6, M7) pre-leukemic marrow disorders myelofibrosis myelodysplasia refractory anemia with excess blasts T-cell leukemia plasmacytoma **osteosarcoma/bone** (7.7%, n = 19) osteoblastic osteosarcoma fibroblastic osteosarcoma extra-skeletal/soft tissue osteosarcoma osteochondroma	**non-melanoma skin cancer** (4.9%, n = 12) squamous cell carcinoma basal cell carcinoma **gastro-intestinal** (4.9%, n = 12) esophageal carcinoma gastric carcinoma pancreatic carcinoma **uterus/ovary** (4.1%, n = 10) ovarian cystadenocarcinoma uterine carcinoma uterine leiomyoma **hepatobiliary** (4.1%, n = 10) cholangiocarcinoma hepatocellular carcinoma **genito-urinary** (3.7%, n = 9) ureteral transitional cell carcinoma bladder transitional cell carcinoma vulvar carcinoma prostate carcinoma **head and neck neoplasms** (3.3%, n = 8) nasal carcinoma NOS hard/soft palate squamous cell carcinoma tongue squamous cell carcinoma laryngeal carcinoma **breast carcinoma** (2.8%, n = 7) **lung** (2.0%, n = 5) squamous cell carcinoma adenocarcinoma bronchioloalveolar carcinoma carcinoid **CNS** (2.0%, n = 5) astrocytoma spinal cord hemangiolipoma **adrenal cortical** (1.6%, n = 4) carcinoma pheochromocytoma

* includes all patients in the study population (see Table 1 and Supplementary Spreadsheet 1).

Table 3: Distribution of neoplasm types between WS patients residing in Japan vs. WS patients residing outside of Japan (1939-2011)

	WS cases residing in Japan	WS cases residing outside of Japan	p-value (Fisher's exact)
Neoplasm type			
soft tissue	19	5	0.63
bone	13	6	0.59
melanomas	28	4	0.082
hematologic/lymphoid	17	6	1
thyroid	35	4	0.016
meningioma	17	10	0.17
other	53	29	
total	**182**	**64**	**0.022***

* indicates significant at α=0.05.

Table 4: Multiple primary neoplasms in Werner syndrome patients

Case	Sex	WS Dx confidence	Tumor diagnosis – type and site (age at diagnosis)****	Ref. #
4	M	probable	osteosarcoma of left tibia (45), malignant melanoma of left foot, plantar surface (45), gastric adenocarcinoma(45), leiomyosarcoma of lung (45), papillary carcinoma of thyroid* (45)	(45)
6	M	possible	meningioma (~34), myelofibrosis (41), follicular adenomas of thyroid* (41)	(46)
19	M	definite	leiomyosarcoma of left bicep (40), follicular adenoma of thyroid* (~40)	(47)
20	F	probable	transitional cell carcinoma of bladder (38), malignant melanoma of nasal cavity (45)	(48)
23	F	probable	papillary carcinoma of thyroid (~47), osteosarcoma of right lower leg (~49)	(49)
25	F	probable	uterine cancer NOS (34), follicular carcinoma of thyroid (36), osteosarcoma of left knee (~37)	(50)
27	F	probable	malignant melanoma of nasal cavity (~45), malignant melanoma of right foot, 5th toe (46)	(51)
30	F	unknown	malignant melanoma of: right large pudental lip (~44), left foot, plantar surface , near toe and near heel (~44)	(52)
33	F	probable	papillary carcinoma of thyroid (~32), AML FAB M4, after RAEB-t/MDS (40)	(53)
46	F	unknown	nasal cancer NOS (age uk), uterine cancer NOS (age uk)	(54)
49	M	definite	thyroid neoplasm NOS (27), papillary carcinoma of thyroid (46), meningioma (46)	(55)
64	M	possible	meningioma (62), MDS (62)	(56)
69**	F	unknown	follicular carcinoma of thyroid (44), thyroid adenoma NOS (44)	(57)
70	M	definite	thyroid neoplasm NOS (43), malignant peripheral nerve sheath tumor of right knee (~47), undifferentiated pleomorphic sarcoma of right thigh (50), osteosarcoma of right calcaneal region (57)	(58)
76	F	probable	uterine myoma (29) leiomyoma of left lower abdomen (~34), cystadenocarcinoma of right ovary (37), thyroid adenoma NOS* (37)	(59)
80	M	probable	thyroid adenoma NOS (48), bronchiolo-alveolar adenocarcinoma of lung (52)	(60)
82	M	definite	pharyngeal cancer NOS (<52), adenocarcinoma of lung (52)	(61)
83	M	probable	fibrosarcoma of unknown site (~44), meningioma (~44)	(62)
85	M	possible	SCC of median soft palate (69), SCC of left edge of tongue (74), SCC of right edge of tongue (75), SCC of hard palate (79), SCC of esophagus (79), transitional cell carcinoma of right ureter (82)	(63)
91	M	probable	follicular carcinoma of thyroid (51), retroperitoneal leiomyosarcoma (51)	(64)
93	M	unknown	bone neoplasm NOS of femur (38), thyroid carcinoma NOS (39), benign fibrous histiocytoma of femur (53)	(65)
95**	M	definite	glioblastoma (26), thyroid neoplasm NOS (26)	(66)
97	M	probable	osteosarcoma of right lower leg (50), malignant melanoma of right conjunctiva (52)	(67)
98	M	unknown	acral lentiginous melanoma *in situ* of right thumb (~21), malignant melanoma of nasal cavity (~31)	(68)
108**	F	probable	papillary carcinoma of thyroid (22), thyroid adenoma NOS (39)	(69)
111	F	probable	lobular breast carcinoma (26), gastric adenocarcinoma (~28)	(70)
112	F	probable	thyroid adenoma NOS (35), SCC *in situ* of right thigh (54)	(71)
116	F	definite	uterine myoma (42), meningioma (42)	(72)
119	M	definite	meningioma (53), malignant melanoma of nasal mucosa (56)	(73)
132	F	definite	adrenal cortical carcinoma (50), papillary transitional cell carcinoma of right ureter (50)	(74)
141	M	unknown	osteosarcoma of right leg (52), meningioma (53)	(75)
142	F	definite	meningioma (56), benign thyroid neoplasm NOS (56)	(76)
300	M	probable	follicular thyroid neoplasm NOS (35), malignant melanoma of right heel (42), acral lentiginous melanoma of left foot, plantar surface (44)	(77)
349	M	definite	follicular/clear cell carcinoma of thyroid (34), AML FAB M6 (erythroleukemia) (34)	(78)
161	M	probable	malignant melanoma of finger (<44), AML FAB M2 (44)	(43)

Table 4: Multiple primary neoplasms in Werner syndrome patients (cont.)

Case	Sex	WS Dx confidence	Tumor diagnosis – type and site (age at diagnosis)****	Ref. #
168	M	probable	basal cell carcinoma on right tip of nose (52), SCC on scalp and left forearm (57)	(79)
171	uk	unknown	orbital osteosarcoma (age uk), thyroid adenoma NOS (age uk), meningioma* (age uk)	(80)
172	M	possible	fibrosarcoma of mediastinum (46), basal cell carcinoma on nose (x 2) and on upper lip (57)	(81)
180	F	probable	undifferentiated pleomorphic sarcoma of left lateral thigh (40), cystadenocarcinoma of ovary (41)	(82)
183	F	definite	SCC on cheek (52), basal cell carcinoma x 2 on face (52)	(83)
185	F	definite	breast carcinoma NOS (<47), ovarian carcinoma NOS (<47), pancreatic adenocarcinoma of head of pancreas (47), carcinoid of lung* (47),	INU 1010 ***
186	M	definite	hepatocellular carcinoma (42), cortical adenoma of adrenal gland* (42)	(84)
188	F	definite	cholangiocarcinoma (42), meningioma* (42), cortical adenoma of adrenal gland* (42)	(85)
191**	M	possible	acral lentiginous melanoma of left thumb and lentigo maligna melanoma under left ear (56)	(86)
200	F	probable	meningioma (~31), thyroid carcinoma NOS (~49)	(87)

* incidental finding at autopsy.

** excluded from multiples count due to the ambiguity in the case reports between thyroid goiter and adenoma or because multiple tumors at the same organ site

*** unpublished case #INU1010 from the International Registry of Werner Syndrome (personal communication from G. M. Martin and J. Oshima).

**** multiple benign tumors or multiple malignancies of the same histology at the same organ site are counted only once in spectrum/risk estimates.

uk = unknown

MDS = myelodysplasia

SCC = squamous cell carcinoma

NOS = not otherwise specified

AML = acute myelogenous leukemia

FAB = French-American-British classification

Table 5: Standardized proportionate incidence ratio (SPIR) analyses for malignancies in Japan resident Werner syndrome patients relative to Osaka, Japan population (1965-2009, ages 10-69)

Site	Observed (n=134)***	Expected****	SPIR	95% CI
soft tissue	15	0.73	20.7*	(11.6, 34.1)
bone	11	0.95	11.6*	(5.8, 20.8)
melanoma of skin	14	0.25	56.1*	(30.7, 94.2)
leukemia	9	5.25	1.7	(0.78, 3.3)
thyroid	22	2.41	9.1*	(5.7, 13.8)
GI**	15	66.3	0.23*	(0.13, 0.37)

* statistically significant result ($p<0.05$).

****** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

*** total cases at all sites (excluding non-melanoma skin cancers) includes benign meningiomas diagnosed prior to 1988.

**** obtained using *CI5* volume case data (i.e., representative sample from 1970-2002).

Table 6: Standardized incidence ratio (SIR) analyses for malignancies in Japan resident Werner syndrome patients relative to Osaka, Japan population (1965-2009)

Tumor	Observed	Expected	SIR	95% CI
soft tissue	15	0.35	42.5*	(23.8, 70.0)
bone	11	0.33	33.1*	(16.5, 59.2)
melanoma of skin	14	0.17	83.2*	(45.5, 139.6)
leukemia	9	2.47	3.6*	(1.7, 6.9)
thyroid	22	1.57	14.0*	(8.8, 21.3)
meningioma	15	0.28	54.2*	(30.4, 89.4)
GI**	16	51.81	0.31*	(0.18, 0.50)
All sites (excluding non-melanoma skin)* **	138	103.81	1.3*	(1.1, 1.6)

* statistically significant result ($p<0.05$).

****** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

*** includes benign meningiomas diagnosed prior to 1988.

note: analysis conditioned on a *WRN* pathogenic allele frequency of $q = 0.0037$

References

1. Epstein CJ, Martin GM, Schultz AL, Motulsky AG. Werner's syndrome: A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine (Baltimore).* 1966;45(3):177-221.
2. Goto M. Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. *Mech Ageing Dev.* 1997;98(3):239-254.
3. Huang S, Lee L, Hanson NB, et al. The spectrum of WRN mutations in Werner syndrome patients. *Hum Mutat.* 2006;27(6):558-567.
4. Monnat RJ, Jr. Cancer pathogenesis in the human RecQ helicase deficiency syndromes. In: Goto M, Miller RW, editors. *From Premature Gray Hair to Helicase - Werner Syndrome: Implications for Aging and Cancer*. Tokyo: Japan Scientific Societies Press; 2001:83-94.
5. Goto M, Miller RW, Ishikawa Y, Sugano H. Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiol Biomarkers Prev.* 1996;5(4):239-246.
6. Monnat RJ, Jr. Werner syndrome. In: Fletcher C, Unni K, Mertens F, editors. *WHO/IARC Monograph on Pathology and Genetics of Tumours of Soft Tissue and Bone*. Lyon: IARCPress; 2002:273-274.
7. Fritz A, Percy C, Jack A, et al., editors. *International Classification of Diseases for Oncology*. 3rd ed. Geneva: World Health Organization; 2000.
8. Ferlay J, Parkin DM, Curado MP, et al. Cancer Incidence in Five Continents, Volumes I to IX (Available from: http://ci5.iarc.fr) In: IARC CancerBase No. 9 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010.
9. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J, editors. *Cancer Incidence in Five Continents, Vol. VII*. Lyon: IARC; 1997.
10. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, editors. *Cancer Incidence in Five Continents, Vol. VIII*. Lyon: IARC; 2002.

11. Curado MP, Edwards B, Shin HR, et al., editors. *Cancer Incidence in Five Continents, Vol. IX*. Lyon: IARC; 2007.
12. Breslow NE, Day NE. Statistical Methods in Cancer Research: Vol II--The design and analysis of cohort studies. *IARC Sci Publ.* 1987(82):48-79.
13. Schellenberg GD, Miki T, Yu CE, Nakura J. Werner syndrome. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, editors. *Online Metabolic and Molecular Basis of Inherited Disease*. New York: McGraw-Hill; 2001:1-36.
14. Satoh M, Imai M, Sugimoto M, Goto M, Furuichi Y. Prevalence of Werner's syndrome heterozygotes in Japan. *Lancet.* 1999;353(9166):1766.
15. Huang S, Lee L, Hanson NB, et al. The spectrum of WRN mutations in Werner syndrome patients. *Human Mutation.* 2006;27:558-567.
16. Ishikawa Y, Sugano H, Matsumoto T, Furuichi Y, Miller RW, Goto M. Unusual features of thyroid carcinomas in Japanese patients with Werner syndrome and possible genotype-phenotype relations to cell type and race. *Cancer.* 1999;85(6):1345-1352.
17. Monnat RJ, Jr. Unusual features of thyroid carcinomas in Japanese patients with Werner syndrome and possible genotype-phenotype relations to cell type and race. *Cancer.* 1999;86(4):728-729.
18. Monnat RJ, Jr. Human RECQ helicases: roles in DNA metabolism, mutagenesis and cancer biology. *Semin Cancer Biol.* 2010;20(5):329-339.
19. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol.* 2011;7(10):569-580.
20. Caria P, Vanni R. Cytogenetic and molecular events in adenoma and well-differentiated thyroid follicular-cell neoplasia. *Cancer Genet Cytogenet.* 2010;203(1):21-29.
21. Handkiewicz-Junak D, Czarniecka A, Jarząb B. Molecular prognostic markers in papillary and follicular thyroid cancer: current status and future directions. *Mol Cell Endocrinol.* 2010;322(1–2):8-28.

22. Ishihara K, Saida T, Otsuka F, Yamazaki N, Prognosis T, Statistical Investigation Committee of the Japanese Skin Cancer S. Statistical profiles of malignant melanoma and other skin cancers in Japan: 2007 update. *Int J Clin Oncol.* 2008;13(1):33-41.
23. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *New Eng J Med.* 2005;353(20):2135-2147.
24. Turajlic S, Furney SJ, Lambros MB, et al. Whole genome sequencing of matched primary and metastatic acral melanomas. *Genome Res.* 2012;22(2):196-207.
25. Romano E, Schwartz GK, Chapman PB, Wolchock JD, Carvajal RD. Treatment implications of the emerging molecular classification system for melanoma. *Lancet Oncol.* 2011;12(9):913-922.
26. Feinberg AP, Coffey DS. Organ site specificity for cancer in chromosomal instability disorders. *Cancer Res.* 1982;42(8):3252-3254.
27. German J. Bloom's syndrome: XX. The first 100 cancers. *Cytogenet Cell Genet.* 1997;93(1):100-106.
28. Wang LL, Gannavarapu A, Kozinetz CA, et al. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *J Natl Cancer Inst.* 2003;95(9):669-674.
29. Siitonen HA, Sotkasiira J, Biervliet M, et al. The mutation spectrum in RECQL4 diseases. *Eur J Hum Genet.* 2009;17(2):151-158.
30. Birch JM, Blair V, Kelsey AM, et al. Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. *Oncogene.* 1998;17(9):1061-1068.
31. Birch JM, Alston RD, McNally RJ, et al. Relative frequency and morphology of cancers in carriers of germline TP53 mutations. *Oncogene.* 2001;20(34):4621-4628.
32. Alter BP. Cancer in Fanconi anemia, 1927-2001. *Cancer.* 2003;97(2):425-440.
33. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood.* 2003;101(3):822-826.

34. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. *Blood.* 2009;113(26):6549-6557.
35. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood.* 2012;119(16):3815-3819.
36. Dror Y. Shwachman–Diamond syndrome: implications for understanding the molecular basis of leukaemia. *Expert Rev Mol Med.* 2008;10:e38.
37. Huang J, Shimamura A. Clinical spectrum and molecular pathophysiology of Shwachman-Diamond syndrome. *Curr Opin Hematol.* 2011;18(1):30-35.
38. De Vos M, Hayward BE, Charlton R, et al. PMS2 mutations in childhood cancer. *J Natl Cancer Inst.* 2006;98(5):358-361.
39. Fukuchi Ki, Tanaka K, Kumahara Y, et al. Increased frequency of 6-thioguanine-resistant peripheral blood lymphocytes in Werner syndrome patients. *Hum Genet.* 1990;84(3):249-252.
40. Kyoizumi S, Kusunoki Y, Seyama T, Hatamochi A, Goto M. In vivo somatic mutations in Werner's syndrome. *Hum Genet.* 1998;103(4):405-410.
41. Moser MJ, Bigbee WL, Grant SG, et al. Genetic instability and hematologic disease risk in Werner syndrome patients and heterozygotes. *Cancer Res.* 2000;60(9):2492-2496.
42. Monnat RJ, Jr. Werner syndrome as a model of human aging. In: Conn PM, editor. *Handbook of Models for Human Aging*. Amsterdam: Elsevier Academic Press; 2006:961-976.
43. Seiter K, Qureshi A, Liu D, et al. Severe toxicity following induction chemotherapy for acute myelogenous leukemia in a patient with Werner's syndrome. *Leuk Lymphoma.* 2005;46(7):1091-1095.

44. Ogburn CE, Oshima J, Poot M, et al. An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type and homozygous mutants. *Hum Genet.* 1997;101(2):121-125.
45. Tsuchiya H, Tomita K, Ohno M, Inaoki M, Kawashima A. Werner's syndrome combined with quintuplicate malignant tumors: a case report and review of literature data. *Jpn J Clin Oncol.* 1991;21(2):135-142.
46. Noda M, Matsui K, Kitagawa M, Ohta M. Werner's syndrome with myelofibrosis following diphenylhydantoin therapy after meningiomectomy: an autopsy case. *J Am Geriatr Soc.* 1990;38(1):59-61.
47. Shinohara K, Kojima T, Nakagawa S, Ishikawa F, Murakami T. [Autopsy case of Werner's syndrome associated with smooth muscle sarcoma]. *Nippon Rinsho.* 1974;32(4):859-865.
48. Yamada H, Okada E, Kim H, Sakakura Y. [A case of Werner's syndrome associated with bladder and nasal malignancies]. *Jibi Inkoka Tokeibu Geka.* 1991;63(2):139-143.
49. Ichioka S, Tanaka H, Uchiyama K, Yamada A. [A case of Werner's syndrome associated with double cancer]. *Keisei Geka.* 1991;34(9):975-982.
50. Ozawa M, Arita D, Hino M, et al. [A case of Werner's syndrome associated with uterine cancer, thyroid cancer, and osteosarcoma]. *Pharma Medica.* 1990;8(2):137.
51. Uehara T, Sakai Y, Isonokami M, Yamamura T, Okada N, Yoshikawa K. [A case of Werner's syndrome associated with melanoma of the nasal cavity and toe]. *Rinhi.* 1994;48(1):95-98.
52. Shibuya H, Kato A, Kai N, Fujiwara S, Goto M. A case of Werner syndrome with three primary lesions of malignant melanoma. *J Dermatol.* 2005;32(9):737-744.
53. Takemoto Y, Hata T, Kamino K, et al. Leukemia developing after 131I treatment for thyroid cancer in a patient with Werner's syndrome: molecular and cytogenetic studies. *Intern Med.* 1995;34(9):863-867.

54. Mano T, Imamura Y, Mano K, et al. [A case of Werner's syndrome associated with primary hypothyroidism]. *Ronen Igaku.* 1990;28(4):553-556.
55. Akiho H, Takayanagi R, Migita Y, Yamashita T, Nawada S. [A case of Werner's syndrome associated with papillary carcinoma of thyroid and brain tumor]. *Rinsho to Kenkyu.* 1993;70(12):3879-3885.
56. Taketomi K, Goto Y, Arai I, Ichinose M, Kinoshita K. [An autopsy case of Werner's syndrome associated with meningioma and myelodysplastic syndrome]. *To-Nyo-Byo.* 1990;33(2):180.
57. Takeuchi T, Oki S, Shimazu T, Kubo S, Nakazima K, Hoshizaki T. [Two cases of Werner's syndrome associated with diabetes mellitus, malignant tumor, and fatty liver]. *Nihon Naika Gakkai Zasshi.* 1985;74(6):855-856.
58. Nakamura Y, Shimizu T, Ishikawa Y, et al. Triple primary sarcoma in Werner syndrome with a novel mutation. *Rheumatology (Oxford).* 2003;42(6):798-800.
59. Ueno T, Miwa U, Onoe N, et al. [An autopsy case of Werner's syndrome with death from ovarian cancer associated with multiple benign tumors]. *Naika.* 1976;37(3):533-537.
60. Yamanaka A, Hirai T, Ohtake Y, Kitagawa M. Lung cancer associated with Werner's syndrome: a case report and review of the literature. *Jpn J Clin Oncol.* 1997;27(6):415-418.
61. Ohnishi S, Fujimoto M, Oide T, et al. Primary lung cancer associated with Werner syndrome. *Geriatr Gerontol Int.* 2010;10(4):319-323.
62. Nakao Y, Hattori T, Takatsuki K, et al. [Immunologic studies on Werner's syndrome]. *Clin Exp Immunol.* 1980;42(1):10-19.
63. Iguchi H, Takayama M, Kusuki M, et al. A possible case of Werner syndrome presenting with multiple cancers. *Acta Otolaryngol.* 2004(suppl 554):67-70.
64. Takuma E, Naruse R, Arai H, et al. [A case of Werner's syndrome associated with multiple neoplasms]. *Nisseki Igaku.* 1988;40:20.

65. Shinjo K, Asai T, Tsuboi S, Takahashi Y, Ichihara S, Fukaya M. [A case of multiple bone tumor complicated by Werner syndrome]. *J Tokai Bone Soft Tissue Tumors.* 1999;10:17-20.
66. Tonami H, Hamada S, Nishiki T, et al. [Werner's syndrome]. *Rinsho Hoshasen.* 1983;28(12):1479-1485.
67. Ichihashi N, Kamiya H, Takagi H, Kitajima Y, Osada K. [A case of Werner's syndrome associated with malignant melanoma and osteosarcoma]. *Skin Cancer.* 1997;12(3):366-369.
68. Yoshida T, Inoue Y, Kiyohara Y, et al. [A case of malignant melanoma of a nasal cavity associated with Werner's syndrome with chemotherapy with intra-arterial infusion]. *Skin Cancer.* 1998;13(1):61-65.
69. Murakami T, Tajiri A, Murakami N, Noguchi S. [Abnormal glucose tolerance and lipid metabolism in Werner's syndrome]. *Ooitaken Igaku kai Zasshi.* 1988;7(1):71-74.
70. Kurosawa M, Kan M, Naganuma H, Sawai T, Sasano N. [Werner's syndrome associated with breast cancer and gastric cancer]. *Igaku no Ayumi.* 1982;121(12):1057-1067.
71. Tamada K, Nakamura Y, Habe K. [A case of Werner's syndrome associated with Bowen disease]. *Skin Cancer.* 2000;15:258-261.
72. Sugimoto N, Terayama K, Fujioka F, Maeda T. [Four cases of Werner's syndrome with special reference to the orthopedic aspect.]. *Chubu Nippon Seikei Geka Saigai Geka Gakkai Zasshi.* 1991;34(3):786-790.
73. Uehara S, Koike T, Azegami T, Yamazaki T. [Autopsy of a Werner's Syndrome patient who died from malignant melanoma]. *Shinshu Igaku Zasshi.* 2004;52(1):15-20.
74. Takazawa R, Ajima J, Yamauchi A, Goto M. Unusual double primary neoplasia: adrenocortical and ureteral carcinomas in Werner Syndrome. *Urol Int.* 2004;72(2):168-170.
75. Tsurubuchi T, Yamamoto T, Tsukada Y, Matsuda M, Nakai K, Matsumura A. Meningioma associated with Werner syndrome--case report. *Neurol Med Chir (Tokyo).* 2008;48(10):470-473.

76. Nakamura Y, Shimizu T, Ohigashi Y, Itou N, Ishikawa Y. Meningioma arising in Werner syndrome confirmed by mutation analysis. *J Clin Neurosci.* 2005;12(4):503-506.
77. Kobori S, Seno J, Kato H, Kitajima Y, Sakamoto Y. [A case of Werner's syndrome associated with malignant melanoma]. *Rinsho Hifuka.* 1987;29(7):691-696.
78. Maruyama M, Kaneko K, Kurokawa I, Arai O. [A case of Werner Syndrome associated with thyroid cancer and erythroid leukemia]. *Nihon Naika Gakkai Zasshi.* 1987;76(5):762-763.
79. Zalla JA. Werner's syndrome. *Cutis.* 1980;25(3):275-278.
80. Jacobson HG, Rifkin H, Zucker-Franklin D. Werner's syndrome: a clinical-roentgen entity. *Radiology.* 1960;74:373-385.
81. Hrabko RP, Milgrom H, Schwartz RA. Werner's syndrome with associated malignant neoplasms. *Arch Dermatol.* 1982;118(2):106-108.
82. Bjornberg A. Werner's syndrome and malignancy. *Acta Derm Venereol.* 1976;56(2):149-150.
83. Cerimele D, Cottoni F, Scappaticci S, et al. High prevalence of Werner's syndrome in Sardinia: description of six patients and estimate of the gene frequency. *Hum Genet.* 1982;62(1):25-30.
84. Oppenheimer BS, Kugel VH. Werner's syndrome: report of the first necropsy and of findings in a new case. *Am J Med Sci.* 1941;202:629-642.
85. Rogers A. Werner's syndrome: report of case with unusual complication. *J Fla Med Assoc.* 1959;46(4):436-438.
86. Barnett JH, Lee FG, Rinsky MJ. Acral lentiginous melanoma and lentigo maligna occurring in Werner's syndrome. *Cutis.* 1983;32(3):277-283.
87. Runne U. [Werner's syndrome with metastasizing malignant struma]. *Z Hautkrankheiten.* 1975;50(19):817-819.

Supplementary Materials

1. Supplementary Tables

Supplementary Table 1: Werner syndrome diagnostic criteria and diagnostic confidence Categories.

Supplementary Table 2: Japan-resident WS patient thyroid histopathologic subtype analysis.

Supplementary Table 3: SPIR sensitivity analysis using only WS cases with highest diagnostic confidence.

Supplementary Table 4: SIR sensitivity analysis conditioned on *WRN* pathogenic allele frequency.

Supplementary Table 5: SIR sensitivity analysis conditioned on WS patient age distribution.

Supplementary Table 6: SIR sensitivity analysis using only WS cases with highest diagnostic confidence.

2. Supplementary spreadsheets:

Supplementary Spreadsheet 1: Study population spreadsheet

Supplementary Spreadsheet 2: Additional case reports not included in the study population

Supplementary Table 1: Werner syndrome diagnostic criteria and diagnostic confidence categories

Diagnostic category	***Sensitivity analysis category***	***Diagnostic criteria****
Definite	High confidence	all cardinal signs + two additional signs ***OR*** confirmed pathogenic mutations in both *WRN* alleles
Probable		first 3 cardinal signs + any 2 others
Possible	Low confidence	either cataracts or dermatological changes + any 4 additional signs
Exclusion**	Exclude	Onset of signs or symptoms before adolescence (except stature)

Diagnostic criteria and categorization notes:

*Cardinal signs and symptoms and diagnostic confidence categories were taken from the diagnostic and categorization criteria of the International Registry of Werner Syndrome: www.wernersyndrome.org/registry/diagnostic.html with the following modifications: 1. putative WS patients with known pathogenic mutations in both WRN alleles were also considered to be 'Definite'/'High confidence'; and 2. "mesenchymal neoplasms, rare neoplasms or multiple neoplasm" was not counted for any of the patients in the determination of Werner syndrome diagnostic confidence.

Reported cataracts were assumed bilateral if not explicitly stated, and characteristic dermatological pathology was considered to be present if any one of the six skin pathologies was reported as defined in the Registry diagnostic signs and symptoms. Where height was provided but not designated as short stature, we classified males with heights <164 cm and females with heights <154 cm as of short stature.

****We excluded no cases on the basis of onset of signs or symptoms prior to adolescence. Two Japan-resident cases were reported with voice changes prior to adolescence, and one Japan non-resident case was reported to have had premature greying of the hair at age 8 (see Supp. Table 2).

Supplementary Table 2: Japan-resident WS patient thyroid histopathologic subtype analysis

Thyroid cancer subtype	**WS cases**	**Osaka population comparison data***	
		ages 0-24	ages 10-69
follicular subtype	11	14	402
papillary subtype	7	102	2,391
other/unspecified type	5	29	583
Total	**23**	**145**	**3,376**
p –value (Fisher's Exact)		*P=0.00002*	*P=0.00001*

* Comparison data from Osaka, Japan population cases reported in *CI5* (vol. 7-9, 1988-2002).

Supplementary Table 3: SPIR sensitivity analysis using only WS cases with highest diagnostic confidence

Site	Observed (n = 88)***	Expected****	SPIR	95% CI
soft tissue	11	0.45	24.4*	(12.2, 43.7)
bone	9	0.59	15.3*	(7.0, 29.0)
melanoma of skin	10	0.15	65.2*	(31.3, 120.0)
leukemia	5	3.33	1.5	(0.49, 3.5)
thyroid	14	1.50	9.3*	(5.1, 15.6)
GI**	7	44.3	0.16*	(0.06, 0.33)

* statistically significant result ($p<0.05$).

** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

*** total cases at all sites (excluding non-melanoma skin cancers) includes benign meningiomas diagnosed prior to 1988.

**** relative to Osaka, Japan population (1965-2009, ages 10-69). Obtained using *CI5* volume case data (i.e., representative sample from 1970-2002).

Supplementary Table 4: SIR sensitivity analysis conditioned on *WRN* pathogenic allele frequency

4A: SIR sensitivity analysis conditioned on a WRN pathogenic allele frequency of q=0.006

Tumor	Observed	Expected**	SIR	95% CI
soft tissue	15	0.93	16.2*	(9.0, 26.6)
bone	11	0.87	12.6*	(6.3, 22.5)
melanoma of skin	14	0.44	31.7*	(17.3, 53.1)
leukemia	9	6.50	1.4	(0.63, 2.6)
thyroid	22	4.12	5.3*	(3.3, 8.1)
meningioma	15	0.73	20.6*	(11.5, 34.0)
GI***	16	136.24	0.12*	(0.07, 0.19)
All sites (excluding non-melanoma skin)**	138	273.0	0.51*	(0.42, 0.60)

* statistically significant result ($p<0.05$).

** relative to Osaka, Japan population, 1965-2009.

*** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

**** includes benign meningiomas diagnosed prior to 1988.

4B: SIR sensitivity analysis conditioned on a WRN pathogenic allele frequency of q=0.0014

Tumor	Observed	Expected**	SIR	95% CI
soft tissue	15	0.05	296.6*	(166.0, 489.3)
bone	11	0.05	231.0*	(115.3, 413.3)
melanoma of skin	14	0.02	581.6*	(318.0, 975.8)
leukemia	9	0.35	25.5*	(11.6, 48.3)
thyroid	22	0.22	98.1*	(61.5, 148.5)
meningioma	15	0.04	378.9*	(212.1, 625.0)
GI***	16	7.4	2.2*	(1.2, 3.5)
All sites (excluding non-melanoma skin)****	138	14.9	9.3*	(7.8, 11.0)

* statistically significant result ($p<0.05$).

** relative to Osaka, Japan population, 1965-2009.

*** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

**** includes benign meningiomas diagnosed prior to 1988.

Supplementary Table 5: SIR sensitivity analysis conditioned on WS patient age distribution

5A: SIR sensitivity analysis with age contraction beginning at age 40 years and a WRN pathogenic allele frequency of q=0.0037

Tumor	Observed	Expected**	SIR	95% CI
soft tissue	15	0.40	37.6*	(21.1, 62.0)
bone	11	0.36	30.3*	(15.1, 54.2)
melanoma of skin	14	0.19	71.9*	(39.3, 120.6)
leukemia	9	2.75	3.3*	(1.5, 6.2)
thyroid	22	1.76	12.5*	(7.8, 19.0)
meningioma	15	0.33	45.9*	(25.7, 75.6)
GI***	16	68.45	0.23*	(0.13, 0.38)
All sites (excluding non-melanoma skin)**	138	131.53	1.1	(0.88, 1.2)

* statistically significant result ($p<0.05$).

** relative to Osaka, Japan population, 1965-2009.

*** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

**** includes benign meningiomas diagnosed prior to 1988.

5B: SIR sensitivity analysis with age contraction beginning at age 20 years and a WRN pathogenic allele frequency of q=0.0037

Tumor	Observed	Expected**	SIR	95% CI
soft tissue	15	0.31	47.9*	(26.8, 79.0)
bone	11	0.31	35.4*	(17.7, 63.4)
melanoma of skin	14	0.14	97.4*	(53.3, 163.5)
leukemia	9	2.19	4.1*	(1.9, 7.8)
thyroid	22	1.35	16.3*	(10.2, 24.6)
meningioma	15	0.23	66.5*	(37.2, 109.6)
GI***	16	37.53	0.43*	(0.24, 0.69)
All sites (excluding non-melanoma skin)**	138	79.01	1.8*	(1.5, 2.1)

* statistically significant result ($p<0.05$).

** relative to Osaka, Japan population, 1965-2009.

*** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

**** includes benign meningiomas diagnosed prior to 1988.

Supplementary Table 6: SIR sensitivity analysis using only WS cases with highest diagnostic confidence

Tumor	Observed	Expected**	SIR	95% CI
soft tissue	11	0.35	31.1*	(15.5, 55.7)
bone	9	0.33	27.1*	(12.4, 51.4)
melanoma of skin	10	0.17	59.4*	(28.5, 109.3)
leukemia	5	2.47	2.0	(0.66, 4.7)
thyroid	14	1.57	8.9*	(4.9, 15.0)
meningioma	10	0.28	36.2*	(17.3, 66.5)
GI***	7	51.81	0.14*	(0.05, 0.28)
All sites (excluding non-melanoma skin)**	89	103.81	0.86	(0.69, 1.1)

* statistically significant result ($p<0.05$).

** relative to Osaka, Japan population, 1965-2009.

*** includes malignancies of the following sites: esophagus, stomach, small intestine, large bowel, liver, gallbladder, and pancreas (ICD-10 C15-25).

**** includes benign meningiomas diagnosed prior to 1988.