

Association of a missense single nucleotide polymorphism, Cys1367Arg of the WRN gene, with the risk of bone and soft tissue sarcomas in Japan

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Bone and soft tissue sarcomas (BSTSs) are rare malignant tumors of mesenchymal origin. Although BSTSs frequently occur in some hereditary cancer syndromes with germline mutations of DNA repair genes, genetic factors responsible for sporadic cases have not been determined. In the present study we undertook a case-control study and analyzed possible associations between the susceptibility to BSTS and the single nucleotide polymorphisms (SNPs) in DNA repair genes. Genomic DNAs extracted from case and control peripheral blood leukocytes were genotyped by pyrosequencing. For candidate polymorphisms, we chose 50 non-synonymous missense SNPs, which we have previously been identified by resequencing 36 DNA repair genes among the Japanese population. In the first screening, we analyzed 240 cases and 685 controls and selected six SNPs at the significance level of $P < 0.1$ (Fisher's exact test). The six SNPs were further analyzed in the second genotyping on an additional set of 304 cases and 834 controls. In the joint analysis (the first and second genotyping combined) of 544 cases and 1378 controls, Cys1367Arg of the WRN gene was found to be a protective factor of BSTS (odds ratio = 0.66, 95% confidence interval = 0.49–0.88, $P = 0.005$). An exploratory subgroup analysis without multiple comparison adjustment suggested that the WRN-Cys1367Arg SNP is associated with soft tissue sarcomas, sarcomas with reciprocal chromosomal translocations and malignant fibrous histiocytoma. (*Cancer Sci* 2008; 99: 333–339)

Bone and soft tissue sarcomas (BSTSs) are nonepithelial, non-hematological malignant tumors of mesenchymal origin. Three characteristics of BSTS are that: (i) they are rare malignancies; (ii) they develop at a variety of sites; any mesenchymal tissues throughout the body; and (iii) show highly heterogeneous histological types. In the United States, the incidence of malignant bone tumors is estimated to be around 0.8 per 100 000 population,⁽¹⁾ and that of soft tissue sarcomas (STS) approximately 5.0 per 100 000.⁽²⁾ It is estimated that 2370 bone and joint malignancies and 9220 malignant soft tissue tumors would newly develop in 2007.⁽³⁾

The rarity and the histological heterogeneity of BSTSs have hampered the identification of the risk factors and etiology. BSTSs show a slight male predominance, and the incidence of these rare tumors both in Japan and in other countries seems to be stable in the last several decades except for an increase in Kaposi sarcoma as reported in the United States.^(4,5) This appears to be in contrast to several other types of cancers, such as gastrointestinal or gynecologic cancers, which have shown a significant change in incidence in Japan probably due to the change in environmental and life style factors. No significant

racial variation has been noted in the overall incidence of sarcomas with some exceptions, such as Ewing sarcoma, which reportedly occurs more frequently in Caucasians.⁽⁶⁾

To date, some environmental and genetic factors for BSTS risk have been suggested. The environmental factors include external radiation therapy,⁽⁷⁾ Thorotrast, arsenical pesticides and medications, phenoxyherbicides, dioxin, vinyl chloride, immunosuppressive drugs, alkylating agents, androgen-anabolic steroids, human immunodeficiency virus, and human herpes virus type 8.⁽⁵⁾ Information on the genetic factors has so far been limited to certain monogenic hereditary cancer syndromes known to be associated with the incidence of BSTS, such as Li–Fraumeni syndrome, hereditary retinoblastoma, and Werner syndrome with a germline mutation of *TP53*, *RBI*, and *WRN*, respectively. These genes are involved in DNA repair and related systems. Other monogenic hereditary syndromes are also associated with the specific type of multiple benign tumors and their malignant transformation, such as multiple neurofibromas and malignant peripheral nerve sheath tumors in neurofibromatosis 1, and multiple osteochondromas and chondrosarcomas in hereditary multiple exostosis, that have a germline mutation of *NFI* and *EXT1/2*, respectively.

Recently, BSTSs have been considered to be divided into two distinct entities based on their somatic genetic aberrations. One group is characterized by reciprocal chromosome translocations resulting in tumor-specific fusion genes, which may be a critical step for pathogenesis. Another group of sarcomas tend to show complex abnormalities in the karyotypes, suggesting an overall increase in genetic and chromosomal instability. However, the precise mechanisms of tumorigenesis in both groups remain unclear.

The characteristic increase in the risk of BSTS in inherited diseases caused by germline mutations in the DNA repair and related systems has prompted us to investigate the association of the DNA repair gene polymorphisms with the risk of BSTSs. A case-control study was carried out on 544 cases with BSTS and 1378 controls at 50 non-synonymous coding single nucleotide polymorphisms (cSNPs), which we have identified by resequencing of 36 candidate genes on a Japanese population⁽⁸⁾. Associated studies of common polymorphisms of the DNA repair genes have already been reported in many types of cancer,⁽⁹⁾ but to our knowledge, this study is the first report on BSTS.

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Table 1. Fifty missense single nucleotide polymorphisms (SNPs) in DNA repair genes grouped by their representative pathways

DNA repair gene	Gene name	SNP	Amino acid change
Base excision repair			
<i>PARP-1/ADPRT1</i>	Poly (ADP-ribose) polymerase family, member 1	T2444C	Val762Ala
		A2978G	Lys940Arg
<i>APE1/APEX</i>	APEX nuclease (multifunctional DNA repair enzyme) 1	A395G	Ile64Val
		T649G	Asp148Glu
<i>MBD4</i>	Methyl-CpG binding domain protein 4	G1212A	Glu346Lys
<i>NUDT1</i>	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	G273A	Val83Met
<i>OGG1</i>	8-oxoguanine DNA glycosylase	C2243G	Ser326Cys
<i>XRCC1</i>	X-ray repair complementing defective repair in Chinese hamster cells 1	C685T	Arg194Trp
		G944A	Arg280His
		G1301A	Arg399Gln
Nucleotide excision repair			
<i>ERCC5/XPG</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 5	C3507G	His1104Asp
<i>ERCC6/CSB</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 6	G1275A	Gly399Asp
<i>XPC</i>	Xeroderma pigmentosum, complementation group C	A2655C	Lys822Gln
<i>XPD/ERCC2</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 2	G1615A	Asp312Asn
		A2932C	Lys751Gln
Mismatch repair			
<i>MLH1</i>	mutL homolog 1, colon cancer, non-polyposis type 2	A676G	Ile219Val
<i>MLH3</i>	mutL homolog 3 (<i>Escherichia coli</i>)	C2645T	Pro844Leu
		C2939T	Thr942Ile
<i>MSH2</i>	mutS homolog 2 (E. coli)	C91T	Thr8Met
<i>MSH3</i>	mutS homolog 3 (E. coli)	A3122G	Thr1036Ala
<i>MSH6</i>	mutS homolog 6 (E. coli)	G203A	Gly39Glu
DNA damage response genes			
<i>TP53</i>	Tumor protein p53 (Li-Fraumeni syndrome)	G466C	Arg72Pro
DNA double strand break repair			
<i>BLM</i>	Bloom syndrome	C967T	Thr298Met
		G4035A	Val1321Ile
<i>BRCA2</i>	Breast cancer 2, early onset	A1342C	Asn372His
<i>KIAA0086</i>	DNA cross-link repair 1A	C1867G	His317Asp
<i>LIG4</i>	DNA ligase IV	A2245G	Ile591Val
<i>NBS1</i>	Nijmegen breakage syndrome 1	G605C	Glu185Gln
<i>RAD51L3</i>	RAD51-like 3 (<i>S. cerevisiae</i>)	G501A	Arg126Gln
<i>RAD54L</i>	RAD54-like (<i>S. cerevisiae</i>)	A551G	Lys151Glu
<i>RINT1</i>	RAD50 interactor 1	G33C	Glu4Gln
<i>WRN</i>	Werner syndrome	C2573T	Thr781Ile
		T4330C	Cys1367Arg
<i>XRCC3</i>	X-ray repair complementing defective repair in Chinese hamster cells 3	C1075T	Thr241Met
DNA polymerase			
<i>POLD1</i>	Polymerase (DNA directed), delta 1	G409A	Arg119His
<i>POLH/XPVR/RAD30</i>	Polymerase (DNA directed), eta	A1840G	Lys535Glu
<i>POLII/RAD30B</i>	Polymerase (DNA directed) iota	A2180G	Thr706Ala
<i>POLL</i>	Polymerase (DNA directed), lambda	C1683T	Arg438Trp
<i>POLZ/REV3</i>	REV3-like, catalytic subunit of DNA polymerase zeta (yeast)	C4259T	Thr1146Ile
<i>REV1</i>	REV1 homolog (<i>S. cerevisiae</i>)	T982C	Phe257Ser
		A1330G	Asn373Ser
Other pathways			
<i>FANCA</i>	Fanconi anemia, complementation group A	G827A	Ala266Thr
		G1080A	Arg350Gln
		A1532G	Ser501Gly
		A2457G	Asp809Gly
		C3294T	Ser1088Phe
<i>FANCE</i>	Fanconi anemia, complementation group E	G451T	Arg89Leu
		G1213A	Arg343Gln
<i>FANCF</i>	Fanconi anemia, complementation group F	A983G	Lys324Glu
<i>FANCG/XRCC9</i>	Fanconi anemia, complementation group G	C1382T	Thr297Ile

ADP, adenosine diphosphate;

Table 2. Case and control subjects

		1st screening		2nd genotyping		Joint analysis	
		Case	Control	Case	Control	Case	Control
Gender	Male	143	483	174	492	317	875
	Female	97	202	130	342	227	503
Age	<40	109	113	127	214	236	318
	≥40	131	572	177	620	308	1060
Institutions [†]	NCCH	240	242	127		367	242
	NCCE			19		19	
	KEIO		302	158	507	158	809
	IWT				327		327
	NNH		141				
Total		240	685 [‡]	304	834	544	1378 [‡]

Distributions of gender, age and five institutions where case and/or control subjects were recruited are listed. [†]The 685 controls in the first screening are from our published data (8). [‡]The 141 NNH control data were not available for the joint analysis. KEIO, Keio University Hospital, Tokyo; IWT, Iwata Hospital, Shizuoka; NCCE, National Cancer Center East, Chiba; NCCH, National Cancer Center Hospital, Tokyo; NNH, National Nishigunma Hospital, Gunma.

Table 3. Histological distribution of the cases and subgroup classification

Bone sarcoma		Soft tissue sarcoma		Subgroup	Total (%)
Osteosarcoma	105	Liposarcoma	111	ALL	544 (100.0)
Chondrosarcoma	41	MFH	92	BS/STS	
EWS/PNET	18	Synovial sarcoma	38	BS	190 (34.9)
MFH	11	Leiomyosarcoma	19	STS	354 (65.1)
Chordoma	8	MPNST	19	Translocation	
Adamantinoma	3	Dermatofibrosarcoma protuberance	17	(+)	154 (28.3)
Leiomyosarcoma	2	EWS/PNET	15	(-)	390 (71.7)
MPNST	1	EMC	7	Histopathology	
Fibrosarcoma	1	Osteosarcoma	6	Osteosarcoma	111 (20.4)
	190	Fibrosarcoma	5	MFH	103 (18.9)
		Epithelioid sarcoma	5	Liposarcoma	111 (20.4)
		Rhabdomyosarcoma	6	Others	219 (40.3)
		Angiosarcoma	6		
		Alveolar soft part sarcoma	4		
		Clear cell sarcoma	2		
		Mesenchymal chondrosarcoma	1		
		Hemangiopericytoma	1		
			354		

ALL, all samples; BS, bone sarcomas; EMC, extraskelatal myxoid chondrosarcoma; EWS/PNET, Ewing sarcoma and peripheral neuroectodermal tumor; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; STS, soft tissue sarcomas; Translocation (+), sarcomas with reciprocal chromosomal translocations; Translocation (-), sarcomas without reciprocal chromosomal translocations.

Materials and Methods

Study design, case and control subjects. Genotype data of the 50 SNPs,⁽⁸⁾ in Table 1 from 240 cases and 685 controls were analyzed as the first screening to select those SNPs to be subjected to the second genotyping in the additional 304 cases and 834 controls. In this study, we used a joint analysis,⁽¹⁰⁾ in which the results of the second genotyping were combined with those of the first genotyping (Table 2).

The cases were recruited in three hospitals (National Cancer Center Hospital [NCCH], Tokyo, National Cancer Center East Hospital, Chiba [NCCE], and Keio University Hospital, Tokyo [KEIO]) from January 2004 to March 2005 (Table 2). The patients, all Japanese, were either newly diagnosed as BSTS or had been followed up in an outpatient clinic for the history of BSTS. The cases consisted of various histological subtypes, which were confirmed by histological examination in each hospital (Table 3).

We used 685 control subjects in the first screening, which were the same control population as those used in our previous

study on the same set of SNPs but on a different type of malignancy, lung cancer (Table 2).⁽⁸⁾ Those subjects consisted of 383 non-cancer patients in two hospitals (NCCH and National Nishigunma Hospital, Gunma [NNH] in Table 2) and 302 healthy volunteers in KEIO (Table 2). We did not, however, include the 141 control subjects from NNH (Table 2) in the subsequent joint analysis, because their individual genotyping data have not been published and are unavailable for analyses other than the lung cancer study. In the second genotyping, we analyzed additional samples from 834 people who participated in the health examination programs (KEIO and Iwata Hospital, Shizuoka [IWT] in Table 2). We consider that these subjects were suitable enough as controls for our cases; most of the control subjects in this study were also analyzed in our separate gastric cancer project involving a SNP-based genome scan, and its data suggested little if any population stratification (unpublished data, 2007). Therefore, the control subjects for the joint analysis totaled 1378 people with a criterion of no history of cancer during the study period (Table 2).

Subgroup analysis was carried out on the subgroups defined by Table 3. For the 'sarcomas with reciprocal chromosomal

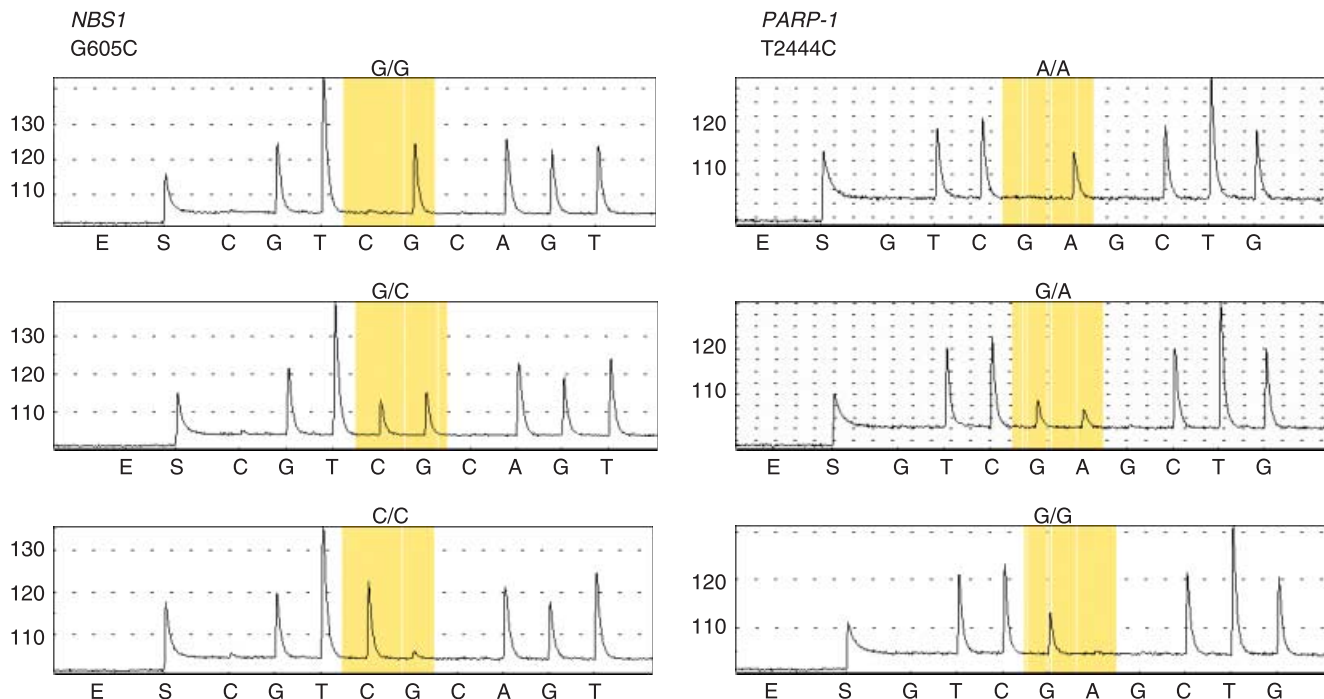


Fig. 1. Genotyping by pyrosequencing. (a) NBS1-Glu185Gln (G605C) and (b) PARP-1-Val762Ala (T2444C) (sequencing in reverse direction). Top, major homozygote; middle, heterozygote; and bottom, minor homozygote.

translocations', we included synovial sarcoma, Ewing sarcoma/peripheral neuroectodermal tumor, myxoid/round cell liposarcoma, clear cell sarcoma, dermatofibrosarcoma protuberance, extraskeletal myxoid chondrosarcoma, and alveolar soft part sarcoma. The specific fusion genes have been reported for those sarcomas, and cytogenetic examinations are routine for their histological diagnosis.⁽¹¹⁾

This study was approved by Institutional Review Board of each institution, and all of the subjects signed an informed consent form to participate in the study.

DNA extraction and genotyping. From each individual we obtained a 10–20-mL sample of whole blood. Genomic DNAs were isolated directly from the samples using Blood Maxi Kit (Qiagen, Tokyo, Japan) or FlexiGene DNA Kit (Qiagen) according to the manufacturer's instructions. Ten nanograms of genomic DNA were subjected to genotyping for 50 SNPs by pyrosequencing using the PSQ96 system (Pyrosequencing, Uppsala, Sweden) as described previously.⁽¹²⁾ Briefly, a genomic fragment containing an SNP site was amplified by polymerase chain reaction (PCR) with a set of PCR primers, one of which was biotinylated. The PCR products were purified using streptavidin-modified paramagnetic beads (Dynabeads M-280; Dynal, Skoyen, Norway), denatured and subjected to nucleotide sequencing by pyrosequencing chemistry. Quality of the SNP typing was confirmed by inspection of the sequence data and by Hardy–Weinberg equilibrium (HWE) tests.

Statistical analysis. Fisher's exact test, odds ratios (OR) and 95% confidence intervals (CI) were used to analyze association of the SNPs and BSTS risk in allele, dominant (i.e. aa + aA vs AA, where 'A' is major allele and 'a' is minor allele) and recessive (i.e. aa vs aA + AA) models.⁽¹³⁾ Crude OR was used for the allele model, while the dominant and recessive models were analyzed using OR adjusted for age (≥ 40 vs < 40 , see Suppl. Fig. S1 online) and gender with 95% CI calculated using a logistic regression analysis. When statistical calculation is not applicable due to 0 subjects being in a cell of a contingency table, we indicate the result as 'NA'.

In order to identify disease-associated SNPs, the following criteria were used: candidate SNPs were selected by *P*-value

< 0.10 on the allele model in the first screening, and then disease associated-SNPs were statistically identified by the allele model in the joint analysis with a multiple comparison adjustment using the Holm's method,⁽¹⁴⁾ for six SNPs. Please note that the family wise error rate may be inflated by a few percent, depending on the number of the true SNPs present in the initial 50 SNPs, by this method of multiple comparison adjustment, because the first and second screenings are not independent in the joint analysis (see Suppl. Table S1 online).

A subgroup analysis was carried out as an exploratory, adjunct analysis without multiple comparison adjustment to address the histological heterogeneity of BSTS. Because the subgroups showed different age preferences, dominant and recessive models were used in order to adjust age and gender by multiple logistic regression.

Results

Results of the joint analysis. The typical pyrosequencing data are shown in Fig. 1, and the results of the two-stage genotyping are summarized in Table 4. Minor allele frequencies and *P*-values of the HWE tests are also listed in Table 4. The six SNPs were selected by the first screening, and the final statistical gene selection was made using all the samples genotyped in the first screening and the second genotyping combined, except 141 controls from Nishigunma Hospital (cases = 544 and controls = 1378 in total). We identified a SNP, WRN-Cys1367Arg, whose allele frequency was significantly different between all BSTS cases and the control subjects (OR = 0.66, 95% CI = 0.49–0.88, *P* = 0.005 and *P* = 0.03, before and after six-SNP multiple comparison adjustment by Holm's method, respectively), showing a protective effect. The minor allele frequency of the SNP is 8.2%.

Subgroup analysis. Since BSTS is characterized by the substantial heterogeneity in its histology, the effects of the DNA repair gene polymorphisms might differ among the subgroups. The results of the subgroup analysis of the WRN-Cys1367Arg SNP are shown in Table 5. Although under-powered and exploratory in nature, the subgroup analysis suggested that the difference in genotype frequency of WRN-Cys1367Arg between the cases and controls

Table 4. Statistics of allele model analysis for the single nucleotide polymorphisms (SNPs) selected in the first screening by $P < 0.1$

Gene	SNP		Minor allele frequency		HWE (P -value)		OR	Allele model	
			Cases (n)	Controls (n)	Cases	Controls		95% CI	P -value
<i>MBD4</i>	G1212A	1st screening	0.304 (240)	0.349 (685)	0.545	0.207	0.82	0.65–1.02	0.083
	Glu346Lys	Joint analysis	0.335 (544)	0.353 (1378)	0.620	0.199	0.92	0.79–1.07	0.286
<i>MSH6</i>	G203A	1st screening	0.275 (240)	0.323 (685)	0.074	0.793	0.80	0.63–1.00	0.057
	Gly39Glu	Joint analysis	0.304 (544)	0.310 (1378)	0.434	0.621	0.97	0.84–1.13	0.750
<i>PARP-1</i>	A2978G	1st screening	0.077 (240)	0.050 (685)	1.000	1.000	1.58	1.04–2.38	0.044
	Lys940Arg	Joint analysis	0.066 (544)	0.052 (1378)	1.000	0.509	1.30	0.97–1.74	0.098
<i>REV1</i>	A1330G	1st screening	0.023 (240)	0.043 (685)	1.000	1.000	0.52	0.27–1.00	0.055
	Asn373Ser	Joint analysis	0.029 (544)	0.044 (1378)	0.042	0.680	0.67	0.45–0.99	0.049
<i>WRN</i>	T4330C	1st screening	0.056 (240)	0.090 (685)	1.000	0.480	0.61	0.40–0.93	0.024
	Cys1367Arg	Joint analysis	0.052 (544)	0.082 (1378)	0.213	0.619	0.66	0.49–0.88	0.005
<i>XRCC1</i>	C685T	1st screening	0.279 (240)	0.327 (685)	0.016	1.000	0.80	0.63–1.00	0.058
	Arg194Trp	Joint analysis	0.292 (544)	0.323 (1378)	0.587	0.811	0.87	0.74–1.01	0.073

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

Table 5. Statistics of subgroup analysis using recessive and dominant models

SNP	Subgroup	(n)	Recessive model [†]			Dominant model [‡]		
			OR	95% CI	P -value	OR	95% CI	P -value
<i>WRN</i>	ALL	544	0.43	0.09–2.01	0.442	0.66	0.48–0.91	0.011
T4330C	BS	190	NA	NA–NA	0.489	0.85	0.53–1.37	0.589
Cys1367Arg	STS	354	0.72	0.16–3.32	0.998	0.58	0.40–0.85	0.005
	Translocation (+)	154	NA	NA–NA	0.632	0.52	0.29–0.95	0.034
	Translocation (–)	390	0.61	0.13–2.86	0.817	0.72	0.51–1.02	0.068
	Osteosarcoma	111	NA	NA–NA	0.826	1.00	0.56–1.80	1.000
	MFH	103	NA	NA–NA	1.000	0.45	0.22–0.94	0.032
	Liposarcoma	111	NA	NA–NA	0.911	0.72	0.40–1.31	0.346

Odds ratios (OR) adjusted for age (≥ 40 , < 40) and gender with 95% confidence intervals (CI) were calculated using a logistic regression analysis for all subgroups. Cases = 544 and Controls = 1378 in total. [†]Recessive model is aa versus aA + AA. [‡]Dominant model is aa + aA versus AA, where a is a minor allele. ALL, all samples; BS, bone sarcomas; MFH, malignant fibrous histiocytoma; NA, statistical calculation not applicable, because the number of the subjects is less than five in any cell in a contingency table; SNP, single nucleotide polymorphism; STS, soft tissue sarcomas; Translocation (+), sarcomas with reciprocal chromosomal translocations; Translocation (–), sarcomas without reciprocal chromosomal translocations.

appears to be significant in STS, sarcomas with reciprocal chromosomal translocations and malignant fibrous histiocytoma. It is noted, however, that the effect direction of the minor allele (Arg1367) of this SNP was protective in all of the subgroups, except osteosarcoma (OR = 1.00), irrespective of the statistical significance. The subgroup analysis data of the other five SNPs analyzed in the second stage is shown in Suppl. Table S2 online.

Discussion

This study attempted the first systematic survey on the possible role of DNA repair gene polymorphisms in the susceptibility to sporadic BSTS. From the joint analysis of the two-stage case-control study on total 544 cases with BSTS of various histology and 1378 controls, a missense SNP of the *WRN* gene, Cys1367Arg, was identified. *WRN* is a member of the RecQ family of DNA helicases, and mutations of the gene can give rise to a rare autosomal recessive genetic instability disorders, Werner syndrome (WS).⁽¹⁵⁾ WS is a premature aging disease characterized by predisposition to cancer and the early onset of symptoms related to normal aging.⁽¹⁶⁾ The types of cancer with elevated risk appear selective, including soft tissue sarcomas, thyroid carcinoma, malignant melanoma, meningioma, hematological malignancies, and osteosarcoma. A diversity of malignancies was found in WS in the literature from 1939 to 1995, but it is noteworthy that the ratio of epithelial to nonepithelial cancers was about 1:1, instead of the usual 10:1.⁽¹⁷⁾ BSTS make up more than 20% of cancer arising in WS patients.⁽¹⁷⁾ Soft tissue sarcomas that have been

identified in WS patients include malignant fibrous histiocytoma, malignant peripheral nerve sheath tumor, fibrosarcoma, rhabdomyosarcoma, liposarcoma, and synovial sarcoma. The reason for the high representation of mesenchymal tumors in WS patients has not been clarified.

WRN encodes a protein with 1432 amino acids that possesses both 3'→5' DNA helicase and 3'→5' DNA exonuclease activities. DNA helicases are enzymes that unwind the energetically stable double-stranded structure of DNA to provide a single-stranded template for important cellular processes such as replication, base excision repair, homologous recombination, and telomere maintenance.^(15,18,19)

Several epidemiological studies have already been carried out on *WRN*-Cys1367Arg. Most of those studies focused on the diseases relating to the WS phenotype, such as myocardial infarction, diabetes mellitus and lymphomas. The more frequent Cys1367 allele has been reported to be associated with a lower frequency of osteoporosis in postmenopausal Japanese women,⁽²⁰⁾ whereas the minor allele Arg1367 may be associated with a lower risk of myocardial infarction and type 2 diabetes mellitus in the Japanese population.^(21,22) With regard to cancer risk, Arg1367 was reported to be associated with a decreased risk of non-Hodgkin lymphoma among women in Connecticut.⁽²³⁾ Of note, in three out of the four reports listed above, *WRN*-Cys1367Arg showed protective effects against the various diseases associated with WS phenotype, and the same held true for our observations on BSTS.

Based on the observations that all of the pathogenic *WRN* mutations identified so far result in truncation of the C-terminal

of the WRN protein, it has been proposed that a lack of the C-terminal nuclear localization signal is important in the pathogenesis of WS.^(24,25) Although WRN-Cys1367Arg is located adjacent to the nuclear localization signal, a previous report has failed to detect any significant difference between WRN (Arg1367) and WRN (Cys1367) with respect to their nuclear localization,⁽²⁶⁾ or helicase and helicase-coupled exonuclease activity.⁽²⁷⁾ Other possible explanations for the observed association include the allelic difference of WRN-Cys1367Arg in the interactions with other proteins or the presence of unknown functionally responsible polymorphisms that are in linkage disequilibrium with WRN-Cys1367Arg.

Some of the SNPs on the genes responsible for a hereditary form of cancer have shown association with a sporadic form of the same type of cancer.^(28–31) Our findings on the WRN SNP on BSTS may add another example of the possible sharing, at least in part, of the oncogenesis pathway between the monogenic and polygenic forms of the same type of cancer and a continuity of the genotype-phenotype spectrum.

It may be controversial to analyze the possible genetic backgrounds with all types of BSTS combined because of its highly heterogeneous nature in histology. However, little if any information is currently available on the genetic predisposition to the

sporadic forms of BSTS, and genetic factors that are common to most BSTSs may exist, as well as those specific to certain subgroups. As one of the first exploratory analyses on the genetic susceptibility of sporadic BSTS, the primary role of this study is to generate hypotheses, which deserve further validation. To validate the hypothesis, evidence should be sought both in statistical replication and meta-analysis using future case-control panels and also in biological functional analyses.

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Supplementary Material

The following supplementary material is available for this article:

Fig. S1. Age distributions of cases and controls in this study. The case appears to have two peaks, before and after around 40 years of age.

A Monte Carlo simulation experiment to compare family wise error rate for the multiple testing correction by six single nucleotide polymorphisms (SNPs) (six hypotheses examined for the joint analysis) and that by 50 SNPs (initial candidate SNPs screened in the first screening). As the framework of simulation, we set the following conditions:

Condition 1.

The total number of SNPs to be examined is set as $m = 50$ and the number of 'true' disease-associated SNPs (positive SNPs) among the 50 SNPs as $N_p = 3$ or 5.

Condition 2.

The population allelic odds ratio for the disease-associated N_p SNPs is $\psi = 1.3, 1.5$ or 1.7, while the population odds ratio for the remaining $m - N_p$ SNPs unrelated to the disease is 1.0.

Condition 3.

In the first stage, sample size is set as 240 cases and 685 control subjects. The second stage sample size is 304 cases and 834 control subjects. In a joint analysis, sample size is set as 544 cases and 989 control subjects.

Condition 4.

The proportion of allele X in the control population is a random variable uniformly distributed in unit interval (0.05, 0.95).

Condition 5.

The criteria to evaluate the performance of each method are two indicators, sensitivity and specificity in the joint analysis.

Condition 6.

The Monte-Carlo simulation to calculate sensitivity and specificity is repeated 10 000 times, and the mean values of indicators are calculated.

Table S1 shows a summary result of the simulation experiment, which suggests that our study was designed to contain the overall false-positive (type 1 error) rate (1-specificity) to 5% or 8% at the power of 90%, if there are five or three true SNPs, respectively, included in our starting 50 candidate SNPs. On the other hand, the false-positive rates for the multiple testing correction by the 50 SNPs with OR = 1.5 or 1.7 are less than 1%.

Table S1. Simulation results for each multiple testing correction method

Table S2. Statistics of subgroup analysis by recessive and dominant models on all the six single nucleotide polymorphisms (SNPs) analyzed in the second stage of genotyping

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