Originals

# *XRCC1* Arg194Trp and *XRCC1* Arg399Gln Polymorphisms Affect Clinical Features and Prognosis of Myelodysplastic Syndromes

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## Abstract

**Backgrounds & Aims**: X-ray repair cross-complementing group 1 (*XRCC1*) plays an important role in base excision repair (BER) system, which is critical for genome maintenance. Polymorphisms in *XRCC1* that result alteration of DNA repair capacity are reportedly associated with cancer risk and treatment response. However, whether these polymorphisms alter the susceptibility and clinical outcomes of patients with myelodysplastic syndromes (MDS) is unknown. The aim of this study was to evaluate the association of two polymorphisms, *XRCC1* Arg194Trp and *XRCC1* Arg399Gln, with susceptibility to and clinical outcome of MDS.

**Methods**: Our study included 119 patients with MDS or chronic myelomonocytic leukemia [median 67.9 years, range 17.1–86.5 years; male/female 81/38] and 202 healthy control subjects. Genotypes were determined via PCR-restriction fragment length polymorphism (PCR-RFLP).

**Results**: Differences in allele or genotype frequencies for *XRCC1* Arg194Trp or *XRCC1* Arg399Gln between patients with MDS and the control group were not significant. However, *XRCC1* 399 non-Arg/Arg genotypes were significantly associated with previous history of radiotherapy and multiple cancers. Furthermore, *XRCC1* 194 non-Arg/Arg genotypes and *XRCC1* 399 Arg/Arg genotype were each significantly associated with poor prognosis for patients with MDS.

**Conclusions**: Our studies suggest that *XRCC1* polymorphisms affected clinical features of MDS and may be useful prognostic marker for MDS.

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## Introduction

Myelodysplastic syndromes (MDS) are a diverse group of clonal hematopoietic stem cell malignancies characterized by profound heterogeneity in morphology, clinical course, and cytogenetic features.[1] Several prognostic scoring systems have been used, including the international prognostic scoring system (IPSS), to evaluate MDS. The cytogenetic feature of MDS is one of the most important prognostic parameters and is incorporated into the IPSS.[2] Recent large-scale genomic studies of MDS have revealed the prognostic significance of mutations in some genes, including *tet methylcytosine dioxygenase (TET2) and additional sex combs like transcriptional regulator 1 (ASXL1)*.[3] These results clearly indicate that mutations play a key role in MDS pathogenesis.

DNA repair pathways play a vital role in maintaining DNA integrity and defects in DNA repair pathways are associated with susceptibility to development of cancer.[4] Furthermore, several findings indicate that altered DNA repair capacity influences the clinical outcome of cancer treatments.[5] The base excision repair (BER) pathway is the main defense mechanism for repairing DNA damage. BER is in-

volved in the repair of base oxidation and DNA single-strand breaks after exposure to reactive oxygen species (ROS), irradiation, or alkylating agents. The human *X-ray repair cross-complementing group1 (XRCC1)* gene encodes a scaffolding protein that plays a crucial role in BER.[6] Moreover, recent reports indicate that XRCC1 plays a role in repair of DNA double-strand breaks.[7] *XRCC1* mutant cells are hypersensitive to DNA damaging agents and display genetic instability after DNA damage.[8] Two common polymorphisms in *XRCC1*, Arg194Trp and Arg399Gln, are reportedly associated with risk for many cancers, including head and neck, esophageal, gastric, breast, lung, and colon cancer.[9,10] Furthermore, several investigators demonstrated that individuals with *XRCC1* 399Gln allele have higher levels of DNA adducts and sister chromatid changes than individuals with *XRCC1* 399Arg allele.[11,12] Thus, these polymorphisms affect the development of cancer by altering efficiency of DNA repair.

Exposure to mutagens, environmental factor, genetic factors, and combinations thereof are associated with an increased risk of MDS.[1] Many previous studies have shown that chemotherapy and radiation are strongly linked to the increased risk of MDS. Inherited genetic defects, including Fanconi anemia, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome are also associated with an increase of MDS or acute myeloid leukemia (AML) in children.[13] Furthermore, mutations in any of several DNA repair genes, including the *glutathione S-transferases multigene1 (GSTM1) or oxoguanine glycosylase 1 (OGG1)* affect MDS risk.[14,15] However, it is unclear whether *XRCC1* polymorphisms alter clinical features of patients with MDS. In our study, we assessed whether either of two *XRCC1* polymorphisms, *XRCC1* Arg194Trp or *XRCC1* Arg399Gln, was associated with risk or clinical outcomes of MDS.

## Methods

### *Patients*

The patient group comprised 119 patients who were diagnosed with MDS or chronic myelomonocytic leukemia (CMML) between August 1993 and October 2013 at Gunma University Hospital and two other cooperative hospitals in Gunma, Japan. The control group comprised 202 healthy volunteers who were recruited as control subjects. Clinical data were collected from medical records of all 119 patients. MDS or CMML was defined according to the World Health Organization (WHO) classification: refractory cytopenia of unilineage dysplasia (RCUD), refractory anemia with ringed sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), refractory anemia with excess of blasts-1 (RAEB-1), refractory anemia with excess of blasts-2 (RAEB-2), myelodysplastic syndrome-unclassified (MDS-U), myelodysplastic syndrome with isolated del5q (5q-), MDS/Myloproliferative neoplasms-unclassi-fiable (MDS/MPN-U), and CMML.[1] IPSS was used to assess prognostic scoring. Cytogenetic subgroups were classified as good: normal, -Y, del (5q), del (20q); intermediate: other abnormalities; poor: complex ($\geq$3 abnormalities) or chromosome 7 anomalies.[2] Each person provided informed consent for their participation. This study was approved by the Institutional Review Board of Gunma University Hospital (Approval number 770).

### *Blood samples and DNA isolation*

Each participant donated 2 mL of whole peripheral blood for the study. QIAmp DNA Blood Midi Kits (Qiagen Sciences, Maryland 20874, USA) were used according to the manufacturer's instructions to extract genomic DNA from whole blood. Quality of purified DNA was assessed spectrophotometrically.

### *Genotyping*

The PCR-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype *XRCC1* Arg194Trp and Arg399Gln, the two *XRCC1* SNPs, as previously described.[16] PCR primers for *XRCC1* codon194 (forward 5'-GCCCCGTCCCAGGTA-3' and re-verse 5'-AGCCCCAAGACCCTTTCACT-3') were used to generate a 497-bp product containing the polymorphic site. PCR primers for *XRCC1* codon 399 (forward 5'-TTGTGCTTTCTCTGTGTCCA-3' and re-verse 5'-TCCTCCAGCCTTTTCTGATA-3') were used to generate 615-bp product containing the polymorphic site. Each PCR reaction was carried out in a total volume of 20$\mu$L. Each reaction mixture contained about 100 ng genomic DNA, 0.2$\mu$M of each primer, 0.2 $\mu$M dNTPs, and 0. 025U/ml of Taq polymerase (TaKaRa Bio, Japan). The PCR conditions for Arg194 Trp reactions were as follows: denaturation at 95°C for 1 minute, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 65°C, and 30 seconds at 72°C. The PCR conditions for Arg399Gln reactions were as follows: denaturation at 95°C for 1 minute, followed by 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The 497-bp PCR products for Arg194Trp were digested with 2 U of MspI (New ENGLAND BioLabs, Japan) at 37°C for 60 minutes and were electrophoresed through 2% agarose gels. MspI digestion of Arg194Trp products resulted in two fragments of 292 bp and 174 bp for homozygous Arg/Arg genotypes, three fragments of 313 bp, 292 bp, and 174 bp for heterozygous Arg/Trp genotypes, and one fragment of 313 bp for homozygous Trp/Trp geno-types. MspI digestion of Arg399Gln products resulted in one fragment of 615 bp for homozygous Arg/Arg genotypes, three fragments of 615 bp, 374 bp, and 241 bp for heterozygous Arg/Gln genotypes, and two fragments of 374 bp and 241 bp for homozygous Gln/Gln genotypes. To confirm the accuracy of PCR-RFLP, amplification products of several individuals were sequenced using an ABI Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

**Table 1** Clinical characteristics of patients with MDS

| | | | |
|---|---|---|---|
| Number of patients | | | 119 |
| Male/Female | | | 81/38 |
| Age, median (range) | | 67.9 | (17.1–86.5) |
| Classification, n (%) | RCUD | 40 | (33.6) |
| | RARS | 6 | (5.0) |
| | MDS-U | 13 | (10.9) |
| | 5q– | 2 | (1.7) |
| | RCMD | 21 | (17.6) |
| | RAEB–1 | 14 | (11.8) |
| | RAEB–2 | 12 | (10.1) |
| | CMML | 10 | (8.4) |
| | MDS/MPN-U | 1 | (0.8) |
| IPSS, n (%) | low | 24 | (20.2) |
| | int–1 | 68 | (57.1) |
| | int–2 | 22 | (18.5) |
| | high | 5 | (4.2) |
| Transfusion, n (%) | | 71 | (59.7) |
| Treatment, n (%) | None | 37 | (31.1) |
| | Supportive care | 42 | (35.3) |
| | CsA | 19 | (16.0) |
| | EPO/G-CSF | 7 | (5.9) |
| | Azactidine | 16 | (13.4) |
| | Chemotherapy | 13 | (10.9) |
| | SCT | 9 | (7.6) |
| Multiple cancers, n (%) | | 28 | (23.5) |
| Previous chemotherapy, n (%) | | 9 | (7.6) |
| Previous radiotherapy, n (%) | | 7 | (5.9) |

IPSS, international prognostic scoring system; CsA, cyclosporin A; EPO, erythropoietin; G-CSF, granulocyte-colony stimulating factor; SCT, stem cell transplantation

### *Statistical analysis*

The frequencies of genotypes and alleles were compared between patients group and control group by using $\chi^2$-tests. Overall survival (OS) was defined as the interval from the date of diagnosis to the date of death or the last follow-up visit. The $\chi^2$-test was used for binary variable comparison. The Mann-Whitney U test was used for continuous variable comparisons. OS was estimated by the Kaplan-Meier method and compared using the log-rank test. $P<0.05$ was considered to indicate statistical significance. SPSS Ver. 5 software or Statflex software ver. 6 was used to perform all calculations.

## Results

### *Clinical characteristics of patients with MDS*

The clinical characteristic of the patients are summarized in Table 1. Of the119 patients, 81 (68.1%) were males, and 38 (31.9%) were female. Median age at diagnosis was 67.9 years (range 17.1–86.5 years). Of the 119 patients, 40 (33.6%) were diagnosed with RCUD, 6 (5.0%) with RARS, 13 (10.9%) with MDS-U, 2 (1.7%) with 5q–, 21 (17.6%) with RCMD, 14 (11.8%) with RAEB–1, 12 (10.1%) with RAEB–2, 10 (8.4%) with CMML, and 1 (0.8%) with MDS/MPN-U. IPSS risk at diagnosis was low for 24 patients (20.2%), intermediate–1 for 68 patients (57.1%), intermediate–2 for 22 patients (18.5%), and high for 5 patients (4.2%). Transfusion was performed on 71 patients (59.7%). Cyclosporine A (CsA) treatment was given to 19 patients (16.0%). Thirteen patients (10.9%) received chemotherapy, and nine patients (7.6%) received allohematopoietic stem cell transplantation (HSCT). In addition, 28 patients (23.5%) had multiple cancers. The most frequent cancer was colon cancer (7 cases, 5.9%) followed by malignant lymphoma (6 cases, 5.0%) and gastric cancer (4 cases, 3.4%). Nine patients (7.6%) had received chemotherapy and seven patients (5.9%) had received irradiation before the diagnosis of MDS.

### *Genotype, allele, and combined genotype frequencies among patients with MDS and among the control group*

The genotype and allele frequencies of the two *XRCC1* SNPs are shown in Table 2. Among the control group, each genotype distribution was in agreement with Hardy-Weinberg equilibrium. The frequencies of genotypes among patients with MDS were as follows: Arg/Arg, 40.3%; Arg/Trp, 48.7%; and Trp/Trp, 10.9% for the Arg194Trp polymorphism and Arg/Arg 57.1%, Arg/Gln, 37.0%; Gln/Gln, 5.9% for the Arg399Gln polymorphism. No significant differences in genotype or allele frequencies were observed between the patients and control groups. Next, we evaluated the patient and control groups with regard to the Arg194Trp-Arg399Gln combined genotype at

**Table 2** Genotype distribution and frequency of *XRCC1* Arg194Trp and *XRCC1* Arg399Gln polymorphisms in patients with MDS and controls

| | MDS (n=119) n (%) | Control (n=202) n (%) | OR (95% C.I.) | P value |
|---|---|---|---|---|
| Arg194Trp | | | | |
| Arg/Arg | 48 (40.3) | 92 (45.5) | Ref. | |
| Arg/Trp | 58 (48.7) | 85 (42.1) | 1.31 (0.81-2.12) | 0.28 |
| Trp/Trp | 13 (10.9) | 25 (12.4) | 1.00 (0.47-2.12) | 0.99 |
| Arg | 154 (64.7) | 269 (66.6) | Ref. | |
| Trp | 84 (35.3) | 135 (33.4) | 1.09 (0.78-1.52) | 0.63 |
| Arg399Gln | | | | |
| Arg/Arg | 68 (57.1) | 116 (57.4) | Ref. | |
| Arg/Gln | 44 (37.0) | 72 (35.6) | 1.04 (0.65-1.68) | 0.87 |
| Gln/Gln | 7 ( 5.9) | 12 ( 5.9) | 1.00 (0.37-2.65) | 0.99 |
| Arg | 180 (75.6) | 304 (75.2) | Ref. | |
| Gln | 58 (24.4) | 96 (23.8) | 1.02 (0.70-1.48) | 0.91 |

OR, odds ratio; 95%C.I., 95% confidence intervals; Arg, arginine; Trp, tryptophan; Gln, glutamine

**Table 3** Combined genotype frequency of *XRCC1* Arg194Trp and *XRCC1* Arg399Gln polymorphisms in patients with MDS and controls

| | MDS (n=119) n (%) | Control (n=202) n (%) | OR (95% C.I.) | P value |
|---|---|---|---|---|
| Arg194Arg/Arg399Arg | 18 (15.1) | 39 (19.3) | Ref. | |
| Arg194Arg/Arg399Gln | 23 (19.3) | 39 (19.3) | 1.28 (0.60-2.73) | 0.53 |
| Arg194Arg/Gln399Gln | 7 ( 5.9) | 14 ( 6.9) | 1.08 (0.37-3.14) | 0.88 |
| Arg194Trp/Arg399Arg | 38 (31.9) | 53 (13.1) | 1.55 (0.78-3.11) | 0.21 |
| Arg194Trp/Arg399Gln | 20 (16.8) | 32 ( 7.9) | 1.35 (0.62-2.98) | 0.45 |
| Arg194Trp/Gln399Gln | 0 ( 0.0) | 0 ( 0.0) | | |
| Trp194Trp/Agr399Arg | 12 (10.1) | 24 (11.9) | 1.08 (0.44-2.63) | 0.86 |
| Trp194Trp/Agr399Gln | 1 ( 0.8) | 1 ( 0.5) | 2.17 (0.14-34.4) | 0.58 |
| Trp194Trp/Gln399Gln | 0 ( 0.0) | 0 ( 0.0) | | |

OR, odds ratio; 95%CI, 95% confidence intervals; Arg, arginine; Trp, tryptophan; Gln, glutamine

**Table 4** Patient characteristics listed according to *XRCC1* Arg194Trp genotype

| Arg194Trp | | Arg/Arg (n=48) | nonArg/Arg (n=71) | P value |
|---|---|---|---|---|
| Male/Female | | 30/18 | 51/20 | 0.28 |
| Age (years), median (range) | | 70.3 (29.3-86.5) | 66.6 (17.1-86.0) | 0.42 |
| Hb (g/dL), median (range) | | 8.5 ( 3.9-19.9) | 10.2 ( 6.3-14.4) | 0.03 |
| Neutrophil ($\times 10^9$/L), median (range) | | 1.3 ( 0.2-13.8) | 1.6 ( 0.2-16.1) | 0.59 |
| Plt ($\times 10^9$/L), median (range) | | 72 (2-429) | 96 (8-594) | 0.05 |
| LDH>WNL, n (%) | | 98 (8-594) | 30 (42.3) | 0.22 |
| RAEB-1 or -2, n (%) | | 9 (18.8) | 17 (23.9) | 0.50 |
| IPSS | int-2 or high, n (%) | 10 (20.8) | 17 (23.9) | 0.69 |
| Cytogenetics adverse risk, n (%) | | 7 (14.6) | 7 ( 9.9) | 0.46 |
| Evolution to leukemia, n (%) | | 6 (12.5) | 16 (22.5) | 0.17 |
| Treatment | None, n (%) | 16 (33.3) | 21 (29.6) | 0.66 |
| | Chemotherapy, n (%) | 3 ( 6.3) | 10 (14.1) | 0.18 |
| | SCT, n (%) | 3 ( 6.3) | 6 ( 8.5) | 0.66 |
| Multiple cancers, n (%) | | 11 (22.9) | 17 (33.3) | 0.86 |
| Previous chemotherapy, n (%) | | 3 ( 6.3) | 6 (11.8) | 0.64 |
| Previous radiotherapy, n (%) | | 2 ( 4.2) | 5 ( 9.8) | 0.50 |

P value, $P<0.05$ is statistically significant; Arg, arginine; Trp, tryptophan; IPSS, international prognostic scoring system; SCT, stem cell transplantation

*XRCC1* (Table 3). The frequencies of each combined genotype in the patient group were as follows: Arg 194Arg/Arg399Arg in 18 patients (15.1%), Arg194 Arg/Arg399Gln in 23 patients (19.3%), Arg194Arg/ Gln399Gln in 7 patients (5.9%), Arg194Trp/Arg399 Arg in 38 patients (31.9%), Arg194Trp/Arg399Gln in 20 patients (16.8%), Arg194Trp/Gln 399Gln in 0 patient (0%), Trp194Trp/Agr399Arg in 12 patients (10.1%), Trp194Trp/Agr399Gln in 1 patient (0.8%), Trp194Trp/Gln399Gln in 0 patient (0%). No signifi-

**Table 5** Patient characteristics listed according to *XRCC1* Arg399Trp genotype

| Arg399Trp | | Arg/Arg (n=68) | nonArg/Arg (n=51) | P value |
|---|---|---|---|---|
| Male/Female | | 47/21 | 34/17 | 0.78 |
| Age (years), median (range) | | 66.9 (17.1-86.0) | 68.9 (35.1-86.5) | 0.95 |
| Hb (g/dL), median (range) | | 8.5 ( 3.9-19.9) | 10.3 ( 5.3-14.4) | 0.007 |
| Neutrophil ($\times 10^9$/L), median (range) | | 1.6 ( 0.2-16.1) | 1.2 ( 0.2-13.8) | 0.12 |
| Plt ($\times 10^9$/L), median (range) | | 98 (8-594) | 78 (2-429) | 0.18 |
| LDH> WNL, n (%) | | 24 (35.3) | 21 (41.2) | 0.51 |
| RAEB-1 or -2, n (%) | | 15 (22.1) | 11 (21.6) | 0.95 |
| IPSS | int-2 or high, n (%) | 12 (17.6) | 15 (29.4) | 0.13 |
| Cytogenetics adverse risk, n (%) | | 7 (10.3) | 12 (23.5) | 0.06 |
| Evolution to leukemia, n (%) | | 15 (22.1) | 7 (13.7) | 0.25 |
| Treatment | None, n (%) | 17 (25.0) | 20 (39.2) | 0.10 |
| | Chemotherapy, n (%) | 8 (11.8) | 5 ( 9.8) | 0.73 |
| | SCT, n (%) | 3 ( 4.4) | 6 (11.8) | 0.17 |
| Multiple cancers, n (%) | | 8 (11.8) | 20 (39.2) | 0.0006 |
| Previous chemotherapy, n (%) | | 3 ( 4.4) | 6 (11.8) | 0.14 |
| Previous radiotherapy, n (%) | | 0 ( 0.0) | 7 (13.7) | 0.0018 |

P value, $P<0.05$ is statistically significant; Arg, arginine; Gln, glutamine; IPSS, international prognostic scoring system; SCT, stem cell transplantation

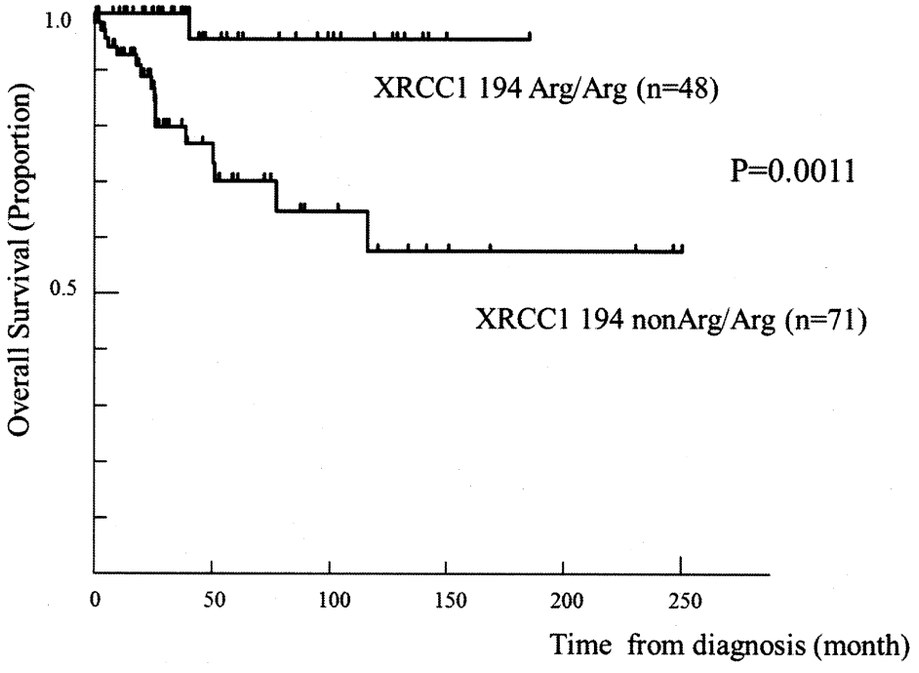


**Fig. 1** Kaplan-Meier overall survival curve in patients with MDS according to *XRCC1* Arg194Trp polymorphism
Among patients with MDS, *XRCC1* 194 nonArg/Arg genotypes were associated with poor prognosis
P=0.0011 log-rank test; Arg, arginine; Trp

cant differences in the distribution of combined genotype were also observed between the MDS patients and the control group.

***The association between XRCC1 Arg194Trp, XRCC1 Arg399Gln genotype and clinical features of MDS patients***

Patient characteristics listed according to *XRCC1* Arg194Trp genotype are shown in Table 4. In the analysis of clinical characteristics, the *XRCC1* Arg/Arg genotype was significantly associated with low Hb level (Arg/Arg: median 8.5g/dL vs. non-Arg/Arg 10.2 g/dL, p=0.03). Other clinical features, including IPSS risk were not significantly different between *XRCC1* Arg/Arg patients and non-Arg/Arg patients. Patient characteristics listed according to *XRCC1* Arg399Gln genotype are shown in Table 5. The *XRCC1* Arg/Arg genotype was also significantly associated with low Hb level (Arg/Arg: median 8.5g/dL vs. non-Arg/Arg 10.3g/dL, p=0.007). Furthermore, *XRCC1* non-Arg/Arg genotypes were significantly associated with previous radiotherapy (non-Arg/Arg:

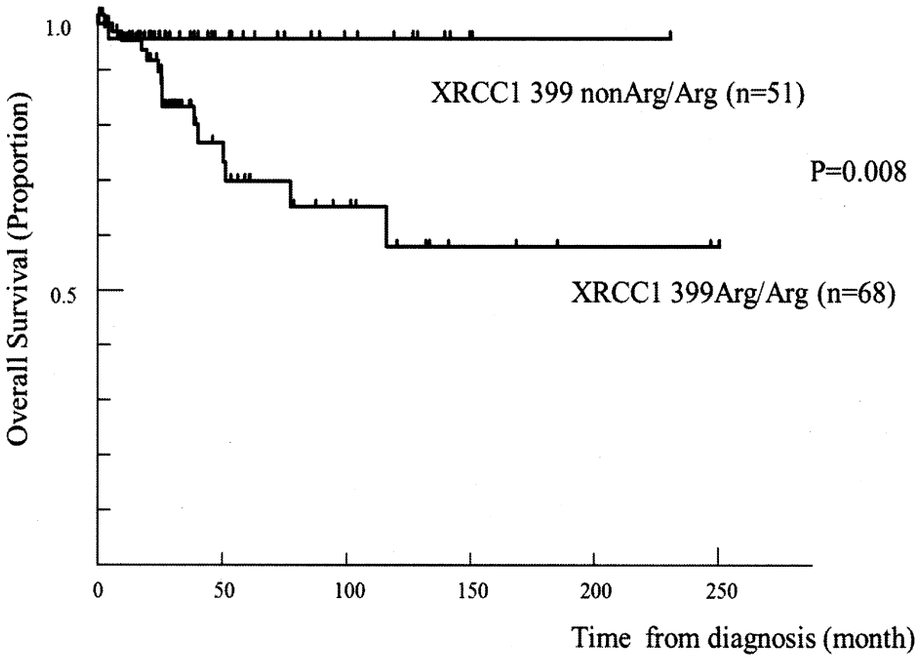


**Fig. 2** Kaplan-Meier overall survival curve in patients with MDS according to *XRCC1* Arg399Gln polymorphism
Among patients with MDS, *XRCC1* 399Arg/Arg genotype was associated with poor prognosis
P=0.008 log-rank test; Arg, arginine; Gln, glutamine

13.7% vs. Arg/Arg: 0% p=0.0018) and multiple cancers (non-Arg/Arg: 39.2% vs. Arg/Arg: 11.8% p=0.0006). However, *XRCC1* non-Arg/Arg genotypes were not significantly associated with previous chemotherapy (non-Arg/Arg: 11.8% vs. Arg: 4.4% p=0.14).

***Survival of patients with MDS according to XRCC polymorphisms***

Survival of MDS patient with respect to genotypes for *XRCC1* Arg194Trp and *XRCC1* Arg399Gln polymorphisms are presented in Figure 1 and 2. *XRCC1* Arg194Trp non-Arg/Arg genotypes were associated with poor prognosis (Fig. 1, p=0.0011). In contrast, *XRCC1* Arg399Gln Arg/Arg genotype was associated with poor prognosis (Fig. 2, p=0.008).

## Discussion

In this study, we investigated the association of two polymorphisms, *XRCC1*Arg194Trp and *XRCC1* Arg399Gln, with susceptibility to and clinical features of MDS in the Japanese population. Our results indicated that neither of the two *XRCC1* polymorphisms was associated with risk of MDS. However, among patients with MDS, *XRCC1* Arg399Gln non-Arg/Arg genotypes were significantly associated with a previous history of radiotherapy and separately of multiple cancers. Furthermore, analysis of OS indicated that *XRCC1* Arg194Trp non-Arg/Arg genotypes and *XRCC1*Arg399Gln Arg/Arg genotype were significantly associated with poor prognosis. These results indicated that these *XRCC1* polymorphisms affect clinical features of MDS.

*XRCC1* is a multifunctional protein that plays an important role in the coordination of two overlapping DNA repair pathways: single-strand breaks repair (SSBR) and BER. ROSs are produced from endogenous and exogenous sources, including ionizing radiation.[9,10] Both oxidized bases and SSBs, which are generated by ROS, are major threats to genetic stability and cell survival; both types of damage accelerate mutation rates and increase levels of chromosomal aberrations. The BER pathway plays a crucial role in the repair of oxidized bases and strand breaks. *XRCC1* has biologically significant interactions with poly (ADP-ribose) polymerase 1 (PARP-1), the gap-filling DNA polymerase $\beta$ (POL$\beta$), the DNA 30-phosphatase (PNKP), and DNA ligase 3a (LIG3a) in the BER pathway.[17] A reduction of *XRCC1* protein levels results in decreased repair capacity and in hypersensitivity to DNA-damaging agents and ionizing radiation.[18] Thus, *XRCC1* is required for efficient SSBR and genomic stability in human cells.

The Arg194Trp polymorphism of *XRCC1* is located in exon 6, which encodes a highly conserved hydrophobic linker region between the POL$\beta$-binding and PARP-1-binding domains; consequently substitution of tryptophan with arginine could alter interactions between *XRCC1* and either of these proteins.[5,9,10,19] Cells with the *XRCC1* 194Arg/Arg genotype show a higher level of DNA damage after exposure to bleomycin or benzo [a] pren-diol-epoxide than do the cells with *XRCC1* 194Trp allele.[20] Furthermore, patients with the *XRCC1* 194Arg/Arg genotype exhibit higher levels of chromosome break than do those with 194Trp allele.[21] However, some conflicting reports state that the patients with *XRCC1* Trp allele show a higher frequency of chromosomal change.

Although numerous studies of polymorphisms report an association between *XRCC1* 194Arg/Trp and cancer risks, the role of *XRCC1* 194Arg/Trp in the development of cancer remains controversial. A recent meta-analysis of 38 published case-control studies indicates that patients with the *XRCC1* 194Arg/Arg genotype have a higher cancer risk than patients with *XRCC1* 194 non-Arg/Arg genotypes.[9,10] Our findings indicated that the *XRCC1* 194Arg/Trp polymorphism was not associated with the susceptibility to MDS.

The Arg399Gln polymorphism of *XRCC1* is located in region encoding the BRCA1 C Terminus (BRCT) domain, which is critical for binding PARP -1. PARP-1 rapidly binds to DNA SSBs and is thereby activated. The poly (ADP-ribosyl) ation of target proteins by activation of PARP-1 results in accumulation of DNA-repair proteins to sites on the damaged DNA. The mutation of the *XRCC1* BRCT I domain showed the reduction of physical interaction with PARP-1. Furthermore, the mutation of the BRCT domain in *XRCC1* prevents SSBR and cell survival after treatment of methyl meth-anesulfonate (MMS).[22] The individual with *XRCC1* 399Gln allele significantly elevated level of sister chromatid exchange in peripheral blood lymphocytes after in vitro exposure to the tobacco-specific nitro-samine 4 -(methylnitrosamino)-1-(3-pyridyl)-1-bu-tanone (NNK).[23] Furthermore, the healthy workers with the *XRCC1* 399Gln allele showed significant increases of baseline DNA damage measured as tail moment by comet assay.[24] These results showed that *XRCC1* 399Gln allele has an impairment of DNA repair capacity.

A meta-analysis of 44 breast cancer case-control studies shows that Asians patients with the *XRCC1* 399Gln allele have a higher risk of breast cancer (OR= 1.54, 95% CI: 1.18-2.01).[25] In addition, a meta-analysis of 19 leukemia case-control studies shows that *XRCC1* Arg399Gln was associated with a higher risk of acute lymphoblastic leukemia.[26] Furthermore, some investigators shows that *XRCC1* 399Gln/Gln genotype is associated with the risks of acute myeloid leukemia (AML).[27,28] However, our results indicated that Arg399Gln was not associated with overall MDS risks.

The most important finding in this study was that MDS patients with *XRCC1* 399 non-Arg/Arg genotypes (Arg/Gln or Gln/Gln) were more likely to have a previous history of radiation than the Arg/Arg patients. Exposure to ionizing radiation is associated with many cancers, including thyroid cancer, breast cancer, and leukemia as well as MDS. Ionizing radiation induces oxidative base damages and SSBs, both of which are mainly repaired by two pathways: BER and homologous recombination repair pathways.[29] Thus, defective repair activity of two pathways may contribute to ionizing radiation sensitivity and increased cancer risk. Many investigators have shown that the *XRCC1* Arg399Gln polymorphism influences cellular response to irradiation. The individual with *XRCC1* 399Gln/Gln genotype shows increased ionizing radiation susceptibility compared to the other genotypes as measured by prolonged cell cycle G2 delay. The workers with the *XRCC1* 399Gln/Gln genotype who were exposed to irradiation have a higher frequency of micronuclei than do workers with the other genotypes.[18] These previous findings are consistent with our data. However, Seedhouse *et al*. reported that *XRCC1* 399Gln/Gln genotype was associated with a protective effect against the development of therapy-related AML.[30] This difference might be partly explained by the definition of therapy-related MDS. We focused on radiation-related MDS. *XRCC1* 399 non-Ar/Arg genotypes was only associated with radiation-related MDS, but not with chemotherapy-related MDS (non-Arg/Arg 13.7% vs. Arg/Arg: 0% p=0.0018, non-Arg/Arg: 11.8% vs. Arg/Arg: 4.4% p=0.14, respectively).

Moreover, our finding indicated that *XRCC1* 399 non-Arg/Arg genotypes were associated with risks of multiple cancers. Many meta-analyses show that *XRCC1* 399Gln/Gln genotype is strongly associated with risk of many types of cancer especially in Asia.[25] Our findings were consistent with the previous finding that the *XRCC1* 399Gln/Gln genotype is associated with low DNA repair activity and risk of many types of cancers.

To our knowledge, the relationship between the polymorphism of *XRCC1* and OS in MDS has not been investigated. Our results showed that *XRCC1* 194 non-Arg/Arg genotypes and *XRCC1* 399Arg/Arg genotype are each associated with poor OS. Recent studies have shown that the efficiency of DNA repair may affect the clinical features of cancers, including treatment response. Among patients with AML, those with higher OGG1 expression had poorer prognoses; OGG1 is a BER protein.[31] Among colorectal cancer patients who received chemotherapy, those with the *XRCC1* 399Arg/Arg or Arg/Gln genotypes had a worse prognosis than that with *XRCC1* 399Gln/Gln.[32] Several investigators also shows the *XRCC1* 399Arg/Arg genotype is significantly associated with poor prognosis in breast cancer patients who are treated with chemotherapy.[5,33] A recent publication has also shown that the *XRCC1* 399Arg/Arg genotype is associated with poor progression free survival in the nasopharyngeal cancer patients who were treated with radiotherapy.[34] Furthermore, *XRCC1* 399Gln/Gln genotype was associated with less aggressive breast cancer. Although conflicting clinical reports exist, some investigators have also reported *XRCC1* 194Trp allele is associated with poor survival in some cancers.[28,35] The possible explanation for our results of OS is that efficient capacity of DNA repair in both *XRCC1* 399 Arg/Arg and *XRCC1* 194 non-Arg/Arg may make resistance to treatment. Although *XRCC1* 194Arg/Arg genotype was associated with lower Hb level, the survival of this genotype was superior to other genotypes. Thus, *XRCC1* 194 geno-type may be an independent prognostic factor. Furthermore, studies

with larger sample sizes are still needed to confirm these associations.

In conclusion, our study showed that *XRCC1* 399 non-Arg/Arg genotype might be associated with radiation-related secondary MDS because the patients with this genotype had a significantly higher incidence of previous history of irradiation for cancers. Furthermore, *XRCC1* 194Arg/Trp and *XRCC1* 399Arg/Gln polymorphisms affected OS. Our finding is agreement with the possibility that effective DNA repair reduces the treatment response. Therefore, *XRCC1* polymorphisms may be associated with pathogenesis and prognosis of MDS.

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## Conflict of Interest and Sources of Funding

The authors state that there are no conflicts of interest relevant to this paper that might bias their work.

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