

Lack of Association of *XRCC1* Codon 399Gln Polymorphism with Chronic Myelogenous Leukemia

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Abstract. *Background:* Recent studies suggest that single nucleotide polymorphisms in different genes may modulate the susceptibility to chronic myelogenous leukaemia (CML). Here, the association of the common *XRCC1* gene polymorphism Arg399Gln at codon 399 in CML was investigated. *Patients and Methods:* Genotyping was performed by melting curve analysis in samples from peripheral blood or bone marrow. *Results:* The frequency of the variant allele 399Gln was similar between the control group and the patients (35.2% and 34.9%, respectively; $p=0.21$). Similarly, the heterozygote and homozygote variant genotypes displayed a homogenous distribution in both groups ($p>0.05$ for all comparisons). Moreover, distribution of the variant allele and subgenotypes did not significantly differ between the patient subgroups with a diagnosis age below or above 50 years. *Conclusion:* To our knowledge, this is the first study to investigate the role of any *XRCC1* polymorphism in CML and our findings do not support a role of codon 399Gln polymorphism in CML.

Chronic myelogenous leukaemia (CML) is a clonal hematopoietic malignancy, characterized by myelogenous hyperplasia. The Philadelphia chromosome, an acquired genetic mutation represented by a translocation between chromosomes 22 and 9, drives the leukemic changes in CML. It represents approximately 14% of all leukemias and most cases of CML occur in adults (<http://www.leukemia-lymphoma.org>). The frequency of the disease increases with age from about one in 1 million in children during the first 10 years of life to nearly two in 100,000 people at age 50, and to one in 10,000 people at age 80 and above. The increase of incidence with age indicates that several steps may be required for genesis of CML, as suggested for many cancer types (1). Although the t(9;22) translocation is the

primary cause, findings of recent studies suggest that single nucleotide polymorphisms in various critical genes may affect the risk of CML. Examples for polymorphic genes associated with increased risk for CML include the *MTHFR* (A1298C) (2), *FLT3* (D324N) (3), *NQO1* (4), *p53* (codon 72) (5), *PDCD5* (6) and cytokine (*TGF-beta*; *IFN-gamma*, *IL4*) genes (7).

DNA repair genes are being increasingly studied in genetic association studies because of their critical role in maintaining genome integrity. Sequence variants in DNA repair genes are thought to modulate DNA repair capacity and consequently have been associated with altered cancer risk (8). The polymorphic gene X-ray repair cross-complementing group 1 (*XRCC1*) may be another candidate for susceptibility in CML pathogenesis. The protein encoded by the *XRCC1* gene, one of more than 20 involved in DNA repair, plays an important role in base excision repair removing single-strand breaks, the most common lesion in cellular DNA (9, 10). It contains a BRCT-1 domain bearing homology to *BRCA1* through which it interacts with DNA ligase III (11) and poly(ADP-ribose)polymerase (12). Of eight nonsynonymous single nucleotide polymorphisms, three are common which lead to amino acid substitutions at codons 194 (exon 6, C to T, Arg to Trp), 280 (exon 9, G to A, Arg to His) and 399 (exon 10, G to A, Arg to Gln). The codon 399 polymorphism is localized in the BRCT-1 domain and has been thus extensively investigated. It has been shown to affect DNA repair capacity (13). Data on the role of the codon 399 polymorphism in cancer susceptibility is not consistent. Although individual studies report an association, especially in Asian populations (14), a meta-analysis (15) comprising 38 case-control studies including breast, esophageal, bladder, gastric and hepatocellular cancers and malignant lymphomas failed to demonstrate an association between codon 399 polymorphism and cancer risk. In lung cancer, the association was found to be modulated by tobacco smoking (15, 16).

The role of *XRCC1* gene polymorphisms in hematopoietic malignancies has rarely been investigated. It was shown that codon 399Gln polymorphism has no effect on cancer risk in

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Table I. Characteristics of the study participants.

	No.	Age range (years)	Gender	Mean age (years)
			Female	Male
Control group	226	18-86	131	95
CML	182	16-75	80	102
AML	72	16-76	40	32
				40.9±13.1

CML: chronic myelogenous leukaemia; AML: acute myelogenous leukemia.

de novo acute myelogenous leukemia (AML), but displayed a protective role in therapy-related AML (17). No data is available on the role and distribution of the codon 399Gln polymorphism in CML. In the present study, we investigated whether the *XRCC1* codon 399Gln polymorphism might affect the risk for CML.

Patients and Methods

The present study was designed to investigate the role of the *XRCC1* codon 399Gln polymorphism in conferring risk for CML. Allele and genotype frequencies were compared among CML patients ($n=182$) carrying the t(9;22) translocation with two groups. The first control group ($n=226$) consisted of healthy individuals without any sign of disease. Another small group ($n=72$) of AML patients served as a further reference for the distribution of the polymorphism. As the incidence of CML increases with age and the mean age of CML patients in our study was about 46 years, the association of the 399Gln polymorphism with the age at diagnosis was also investigated. Patients enrolled in the study were those who were admitted to our institute for a routine translocation analysis. Characteristics of the study participants are given in Table I. The study protocol was approved by the Istanbul Medical Faculty Ethics Committee.

Genomic DNA was prepared from blood lymphocytes according to the standard protocol (18) and used in LightCycler PCR (LC PCR) for genotyping. The reaction was carried out in a LightCycler Instrument (Version 1.2, Roche, Mannheim, Germany). The reaction mixture consisted of 2 μ l Hybridization mix (containing the reaction buffer, Taq polymerase, dNTPs and 10 mM MgCl₂), 3 mM MgCl₂, 0.2 μ M of each primer, 0.2 μ M of each probe, and 2 ml of genomic DNA (1 mg). The primer and probes as reported in Schneider *et al.* (19) were used. Primer sequences were 5'-CCCAAGTACAGGCCAGGTC-3' (sense) and 5'-TGTCCCGCT CCTCTCAGTAG-3' (antisense). Probes were 5'-CCCTCCCAGA GGTAAGGCC-FL and LC640-CACACGCCAACCTGCTCC TTAT-PH. The primer pairs amplify a 238-bp region from exon 10 of the *XRCC1* gene flanking the polymorphic site. The FL-probe covers the polymorphic site and was designed to recognize the polymorphic sequence. Depending on the presence of mismatches between amplicons and the probe, the wild-type and variant sequences were distinguished by melting curve analysis as reported elsewhere (20).

Statistical significance of differences between the allele and genotype frequencies was analyzed using the two-tailed Chi-square test and p -values ≤ 0.05 were considered as statistically significant.

Table II. Codon 399Gln polymorphism in the control and patient groups.

	No.	Arg/Arg	Arg/Gln	Gln/Gln	Gln frequency
Control group	226	96 (42.5%)	101 (44.7%)	29 (12.8%)	0.352
CML	182	76 (41.8%)	85 (46.7%)	21 (11.5%)	0.349
AML	72	27 (37.5%)	36 (50%)	9 (12.5%)	0.375

CML: chronic myelogenous leukaemia; AML: acute myelogenous leukemia.

Table III. Age-distribution of the 399Gln allele in CML patients.

	No.	Arg/Arg	Arg/Gln	Gln/Gln	Gln frequency
Age at CML diagnosis (years)					
≤50	110	51 (46.4%)	46 (41.8%)	13 (11.8%)	0.327
>50	72	27 (37.5%)	37 (51.4%)	8 (11.1%)	0.368

CML: chronic myelogenous leukaemia.

Results

The *XRCC1* gene codon 399Gln polymorphism status was investigated in all samples from study participants. In Figure 1 the melting curves from samples with three different genotypes are displayed. As the fluorescently labeled probe FL was designed to recognize the polymorphic sequence, homozygote variant genotypes displayed a peak at melting temperatures of approximately 63.5°C while, due to a mismatch between the wild-type sequence and the probe, the homozygote wild-type genotype had a lower melting temperature (at approximately 56.5°C). The heterozygotes display both peaks. To confirm the results, LightCycler products of 10 samples were cut by the restriction enzyme *Hpa*II recognizing the wild-type sequence. The results of both approaches were consistent with each other (Data not shown).

Alleles and genotypes are shown in Table II. The allele frequencies in the healthy controls were tested to be consistent with those predicted from the Hardy-Weinberg (HW) equilibrium and indicate that no evolutionary change had occurred in the allele frequency. As shown in Table II, the frequency of the variant allele 399Gln was 35.2% in healthy individuals and 34.9% among the patients ($p=0.21$; Odds ratio (OR) 0.98, 95% confidence interval (CI) 0.74-1.32). This very similar rate was the consequence of the homogenous distribution of the heterozygote and homozygote variant genotypes in both groups ($p>0.05$ for all comparisons). The distribution of the variant 399Gln allele and genotypes was also similar between CML and AML patients ($p>0.05$).

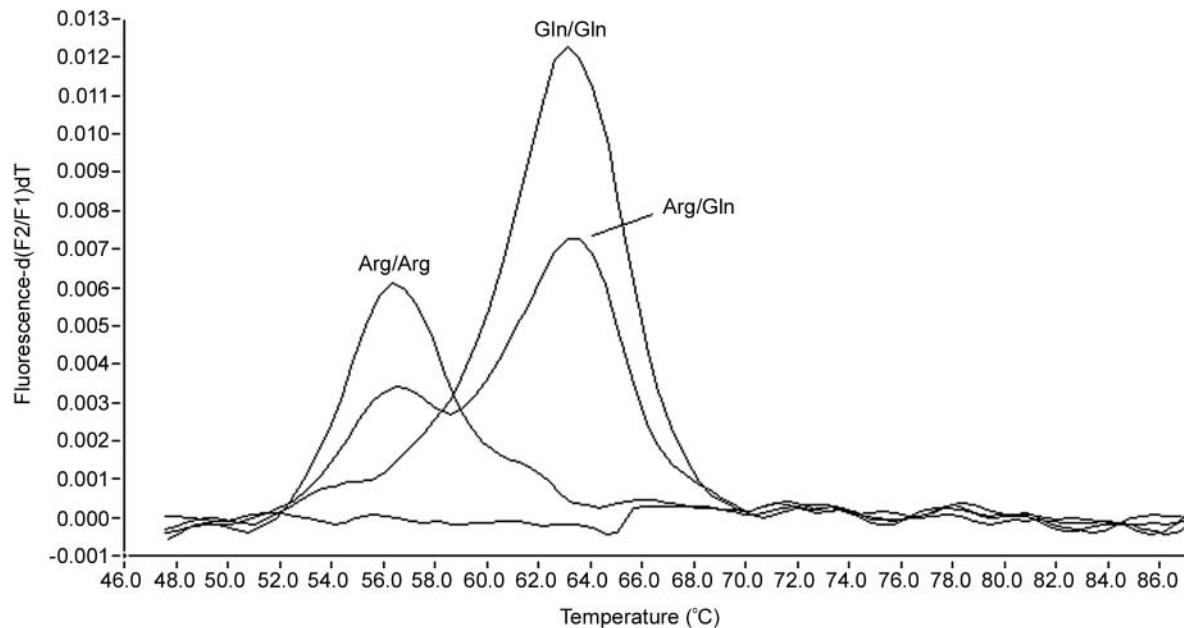


Figure 1. Analysis of the *XRCC1* codon 399Gln polymorphism by melting curve analysis. Following amplification, melting curves were obtained by increasing the temperature gradually from 45°C to 95°C with continuous measurement of the fluorescence. These data were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature (-dF/dT) against temperature. As the fluorescently labelled probe recognized the polymorphic sequence, the homozygote variant genotypes (Arg/Arg) displayed a peak at melting temperatures of approximately 63.5°C while, due to a mismatch between the wild-type sequence and the probe, the homozygote wild-type genotype (Gln/Gln) had a lower melting temperature (at approximately 56.5°C). The heterozygote samples display both peaks. The negative control included water instead of DNA and displayed no peaks.

Since CML risk increases with age, the risk may be different between young and older patients, hence we further analyzed the association of the 399Gln allele with risk of CML in relation to the age at diagnosis. Here, we compared the allele frequency among the subgroup of CML patients diagnosed at age 50 years or younger to the frequency among those with a diagnosis age over 51 years (Table III). Distribution of the variant allele among the subgroup with a diagnosis at 50 years or younger (32.7%) was not significantly different from that of those diagnosed when 51 years or older (36.8%) ($p=0.38$, OR 1.2 95%CI 0.77-1.86). This indicates that *XRCC1* codon 399Gln polymorphism displays a homogenous distribution in relation to the age at diagnosis.

Discussion

Findings of recent genetic association studies suggest that single nucleotide polymorphisms in genes of diverse functions may affect CML risk (2-7). These include genes involved in different cellular pathways including the folate metabolism (*MTHFR*), signal transduction (*FLT3*), detoxification (*NQO1*), immune response (cytokines), genome stability (*p53*) and apoptosis (*PDCD5*). This suggests that in addition to the t(9;22) translocation other

factors may modulate the susceptibility to CML. In the present study, we hypothesized that the codon 399Gln polymorphism of the *XRCC1* gene, an important polymorphic gene involved in base excision repair, might contribute to the CML risk. To date, this is the first study to investigate the distribution of the *XRCC1* codon 399Gln polymorphism in CML.

The allele frequency of 35% in the Turkish population is very similar to that of Caucasians (34%), while this allele seems to be less frequent (about 27%) in Asian populations (14) suggesting ethnical variance. Similar ethnical background in the groups and a positive-translocation status in all CML patients should ensure the homogeneity in our study. The findings of our study show that the variant allele 399Gln and corresponding subgenotypes display a homogenous distribution in all groups and do not provide evidence for any role of codon 399Gln polymorphism in CML susceptibility. The distribution of the variant allele and subgenotypes also did not significantly differ between the patient subgroups with a diagnosis age below or above 50 years, suggesting a homogenous distribution.

Although codon 399Gln polymorphism has been shown to affect the DNA repair capacity of the *XRCC1* protein (13), according to our findings it does not seem to play a

role in the susceptibility for CML. However, larger studies are warranted to confirm this result. In spite of the lack of association between *XRCC1* codon 399Gln polymorphism and CML, many of the afore-mentioned studies reported a positive association between other single nucleotide polymorphisms and CML risk. However, factors such as low statistical power for detecting a moderate effect, false-positive results, heterogeneity across study populations, failure to consider effect modifiers such as environmental exposures, and publication bias should be considered when evaluating the association between single nucleotide polymorphisms and cancer risk (16).

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