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Clinical relevance of *TP53* polymorphic genetic variations in chronic lymphocytic leukemia

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ABSTRACT

Objectives: To analyze the distribution of single nucleotide polymorphisms (SNPs) in the *TP53* gene in chronic lymphocytic leukemia (CLL) patients and to evaluate their associations with clinical behavior of the disease.
Methods: SNPs in exons and parts of adjacent introns of the *TP53* gene were analyzed in 235 CLL patients observed during 2005-2012 years. Data on individuals of European descent from the 1000 Genomes Project data set were used as a reference.
Results: In the recessive model of inheritance, we found borderline associations between CLL risk and C/C genotype of rs1642785 ($p = 0.048$); G/G genotype of rs2909430 (in men only; $p = 0.036$) and Pro72Pro genotype of rs1042522 (in men only; $p = 0.045$). Risk of CLL was increased also in carriers of rare haplotypes ($p = 0.0049$). Besides, genotypes Pro72Pro of rs1042522, C/C of rs1642785, and G/G of rs2909430 were associated with an increased incidence of *TP53* mutations. Median of overall survival in rs1800372 carriers was comparable to that of patients with *TP53* mutations. Other evaluated SNPs were not associated with survival.
Conclusion: Our data suggest that some *TP53* variants may affect the risk of CLL. rs1800372 polymorphism might be the marker of unfavorable prognosis of the disease.

1. Introduction

The *TP53* gene encodes p53 which plays a central role in cell cycle arrest and apoptosis following DNA damage [1,2]. At present, over 200 single nucleotide polymorphisms (SNPs) in *TP53* have been identified (http://www-p53.iarc.fr/). Some of them have been suggested to contribute to the susceptibility to different types of cancer and that they may have additive effects on clinical outcome [3–6]. The vast majority of studies investigated rs1042522, which results in p53 amino acid 72 change from arginine to proline (*TP53* Arg72Pro). According to Dong et al. [7], the *TP53* Pro72 allele potentially increases the prognostic significance of *TP53* mutations in chronic lymphocytic leukemia (CLL). Associations of other *TP53* SNPs with cancer risk in the general populations and clinical behavior of tumors have been addressed only in a few studies [8–12]. Kochethu et al. [13] found that the intron 3 A2/A2 genotype of rs17878362 was strongly associated with early stage of disease, CD38 negativity and a longer time to first treatment in CLL patients. The aim of our work was to analyse the distribution of exonic and intronic SNPs in *TP53* of CLL patients and to evaluate the associations of genetic variants with clinical behavior of the disease.

2. Material and methods

The frequencies of *TP53* SNPs were determined in a series of 235 sequential CLL patients referred to the Research Center for Radiation Medicine, Kiev during 2005 - 2012 years. The study was

Abbreviations: 17p, 17p deletion; CI, confidence interval; CLL, chronic lymphocytic leukemia; HR, hazard ratio; HWE, Hardy–Weinberg equilibrium; IGHV, immunoglobulin heavy chain variable; LD, linkage disequilibrium; M, mutated; OR, odds ratio; OS, overall survival; SNP, single nucleotide polymorphism; TLR2, Toll-like receptor 2; UM, unmutated.

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approved by the local Ethics Review Committee; all patients provided informed consent prior to participation. The diagnosis of CLL was based on clinical history, lymphocyte morphology, and immunophenotypic criteria according to iwCLL guidelines. The group included previously untreated 175 males and 60 females, median age 57 years; Binet stage at diagnosis: A – 121 patients, B – 83 patients, C – 31 patients. The immunoglobulin heavy chain variable (*IGHV)* gene mutational status was assessed as described in our earlier study [14]. Using the 98% threshold for homology to the germ line, 85 (36.2%) patients expressed mutated (M), and 150 (63.8%) patients expressed unmutated (UM) *IGHV* genes.

Genomic DNA for molecular analysis was extracted from peripheral blood mononuclear cells using the QIAamp Blood Mini Kit (Qiagen, Crawley, United Kingdom) according to the manufacturer's protocol. *TP53* genotyping was performed by PCR amplification followed by direct sequencing with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Amplified regions (exons 3-10 and adjacent portions of introns), primers and regimens of amplification are listed in Supplementary Table S1. In addition to 235 patients, CLL patients in whom only part of studied regions of the *TP53* gene were sequenced, were also included in the analysis of SNP frequencies as indicated in Table 1. Obtained data were validated using the IARC *TP53* Mutation Database (http://p53.iarc.fr/) and NCBI dbSNP build 149 database (http://www.ncbi.nlm.nih.gov/SNP).

Considering functional interaction between the p53 fragment encompassing the Arg72Pro region and MDM2, the *MDM2* SNP309 T > G was assessed by PCR-restriction fragment length polymorphism according to Hirata et al. [15]. The functional SNP scoring system (from −2 to +2) proposed by McGraw et al. was used [6]. Patients were stratified into either high (≥0) or low p53 functional scoring groups (<0).

Hardy–Weinberg equilibrium (HWE) was evaluated using the chi–square (χ^2) test. Linkage disequilibrium (LD) between SNPs was estimated by calculating the D' and r^2 measures using CubeX online tool (http://www.oege.org/software/cubex/) and/or SNPstats tool (http://bioinfo.iconcologia.net/snpstats/start.htm). SNPstats was also used for comparison of genotype and haplotype frequencies with those for individuals of European ancestry retrieved from the 1000 Genomes Project dataset (http://www.1000genomes.org/) [16] and for analysis of associations between SNPs and clinical data. Overall survival (OS) was defined as time from diagnosis to the date of death due to any cause or of the last follow-up examination, whichever occurred first, estimated by the method of Kaplan and Meier and assessed by the log-rank test. Univariate and multivariate Cox models were used to test the prognostic significance of each parameter. Model optimization was performed by stepwise backward elimination. All tests were two-sided and considered statistically significant when $p \leq 0.05$. Statistical calculations were performed with SPSS for Windows (version 13.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Association between individual SNPs and CLL risk

As shown in Table 1, eighteen validated SNPs and three potential SNPs were detected in the CLL cohort under analysis. In addition to well-known *TP53* polymorphisms, 4 rare exonic and 7 rare intronic validated SNPs with minor allele frequency (MAF) < 0.05 were found. All validated rare exonic SNPs were silent. Only two SNPs (rs12947788 and rs12951053) exhibited the departure from HWE (p < 0.05). The distribution of most of validated SNPs did not differ significantly from the data on healthy Europeans reported in the literature, except for rs1800370 and rs113530090, whose frequencies tended to be lower in the CLL cohort. In contrast to CLL patients, rs150293825, rs539224556 and rs373232559 were extremely rare in healthy persons (Table 1).

In the recessive model of inheritance, weak but significant association was found between the risk of CLL and C/C genotype of rs1642785 (C/C *vs.* G/C + G/G: OR = 1.73; 95% CI 1.01–295; p = 0.048). There was also a tendency toward an increased frequency of Pro72Pro homozygotes of rs1042522 among CLL patients (Pro72Pro *vs.* Arg72Pro + Arg72Arg: OR = 1.68; 95% CI 0.94–2.87; p = 0.062), and these differences reached the statistical significance when men only were analyzed (OR = 2.52; 95% CI 1.26–5.02; p = 0.045). Similarly, statistically significant association between the G/G genotype of rs2909430 and CLL risk in the recessive model of inheritance was found only in male patients (OR = 4.26; 95% CI 1.14–15.96; p = 0.036).

Besides the validated *TP53* SNPs, three variants were considered to be presumable SNPs, since each of them was represented by two primary CLL cases (i.e., before treatment). Furthermore, in one patient, the c.665C > T substitution was found in DNA from the buccal mucosa as well as in tumor DNA (germline DNA samples were not available for other CLL patients). Two of these substitutions (c.665C > T, rs146340390, and c.672G > A, rs267605076) are specified in the IARC *TP53* database as SNPs and as rare mutations (their frequencies in tumors are 8 of 29893 cases, 0.026% and 9 of 29893 cases, 0.03%, respectively). The third variant, c.1059C > G in exon 10 of *TP53,* has not been previously described. The c.665C > T results in the proline to leucine substitution at position 222 (p.P222L) which strongly impairs p53 activity (23.96% compared to wild-type p53); the other two presumable SNPs are silent.

3.2. Linkage disequilibrium and haplotype association with CLL

SNPs with MAF ≥ 0.05 were used for haplotype analysis. Exonic rs1042522 was in linkage disequilibrium with intronic rs1642785, rs17878362, rs2909430, rs12951053, rs12947788 and rs17883323 (Fig. 1). Above-mentioned SNPs were associated with each other (Supplementary Table S2). Strong association was found between rs12951053 and rs12947788 (D' = 0.9992; r^2 = 0.9992; p < 0.001), and only 3 their combinations were present (81.7% CC/TT; 15.0% CT/CG, and 3.3% TT/GG). Most patients with rare exonic SNPs had no rare intronic SNPs, and *vice versa*. Only rs1800370 and rs145153611, which are in strong LD, were simultaneously found in two patients.

On the analysis of rs1042522, rs1642785, rs17883323, rs2909430, rs12947788 and rs12951053 (rs17878362 was excluded because of the absence of data in the 1000 Genomes Project dataset), a total of 15 haplotypes were identified. Five most frequent haplotypes accounted for 95.64% of CLL cases and 98.44% of healthy European controls. Rare haplotypes occurred more frequently in the CLL group (4.36%) than in healthy individuals (1.56%), resulting in a significant association signal (OR = 3.50; 95% CI 1.60–7.70, p = 0.0018) (Table 2).

3.3. Association with clinical data and second tumors

None of SNPs was associated with sex, age and stage of disease at diagnosis. As for clinical correlations, carriers of A allele of rs17883323 had higher initial leukocytosis ($71.18 \pm 16.96 \times 10^9$/L) comparing with carriers of the C/C genotype ($43.06 \pm 3.89 \times 10^9$/L), p = 0.034. For all genotypes: 234×10^9/L (A/A genotype); $63.02 \pm 15.64 \times 10^9$/L (C/A genotype), and $43.06 \pm 3.89 \times 10^9$/L (C/C genotype), p = 0.002.

The GG genotype of rs2909430 was strongly associated with mutated *IGHV* genes (7 of 9 cases; 77.7%) comparing with 34.5% for other genotypes (p = 0.007).