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Original Article

Influence of polymorphisms of codon 72 and intron 6 of *TP53* gene in patients with chronic lymphocytic leukemia

Martin A. Cabero-Becerra[1,2], Jose A. García-Vela[3], Pedro Sánchez-Godoy[4], Miguel Piris-Villaespesa[5], Belen Navarro-Matilla[1], Nuria Pérez-Sanz[1], Sara Nova-Gurumeta[1], Belén Fernández-Cuevas[1], Angel Arias-Arias[6] and Jose A. Garcia-Marco[1,*]

[1]Molecular Cytogenetics Unit, Hematology Department, Hospital Universitario Puerta de Hierro-Majadahonda and Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana, Madrid, Spain;
[2]Hematology Department, Hospital General La Mancha-Centro, Alcázar de San Juan, Spain;
[3]Hematology Department, Hospital Universitario de Getafe, Madrid, Spain;
[4]Hematology Department, Hospital Universitario Severo-Ochoa, Madrid, Spain;
[5]Hematology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain;
[6]Research Unit, Hospital General Mancha-Centro, Alcázar de San Juan, Spain.

ABSTRACT

Polymorphisms of the *TP53* gene have been studied extensively, and polymorphisms of codon 72 and intron 6 may be the ones most related to the risk of cancer. However, the relationship of these polymorphisms with a worse prognosis in chronic lymphocytic leukemia is unclear. We analyzed these polymorphisms, using real-time polymerase chain reaction and, in many cases, Sanger sequencing, in 558 patients diagnosed with chronic lymphocytic leukemia. The Pro/Pro genotype was significantly associated with Binet stage B and C chronic lymphocytic leukemia, a higher frequency of Richter transformation, and a shorter time to first treatment for their disease from the time of diagnosis. In contrast, the Arg/Pro genotype was associated with a lower risk of secondary neoplasms. Analyses of overall survival demonstrated that patients with the Arg/Pro genotype lived significantly longer than those with the other genotypes (p = 0.028). In particular, the Pro/Pro homozygous genotype at *TP53* codon 72 was identified as an independent variable associated with a 1.7-fold increased risk of death (95% CI: 1.062-2.810; p = 0.028) in comparison with the Arg/Pro heterozygous genotype. These results suggest that the Pro/Pro genotype of *TP53* codon 72 has a potential role in the progression and higher mortality of chronic lymphocytic leukemia patients. Conversely, the Arg/Pro genotype was associated with a lower incidence of secondary malignancies and higher overall survival.

KEYWORDS: chronic lymphocytic leukemia, p53 tumor suppressor protein, gene polymorphism, prognosis.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease where some patients show a relatively stable clinical course, while others present with rapid clinical progression that requires treatment [1].

Many different essential prognostic factors have been identified for CLL, particularly chromosomal abnormalities and the mutational status of IgHV. In addition to the del(17p)/TP53 mutation,

*Corresponding author: jagarciam@aehh.org

the most robust CLL biomarker for response to therapy, other mutations reportedly are correlated with disease outcome; however, they are not yet actionable [1].

Richter transformation (RT) is an aggressive lymphoma in patients with a previous or concomitant CLL diagnosis. Various clinical, genetic, biological, and therapeutic factors are reportedly associated with RT, however, their direct RT pathogenesis correlations remain unexplained [2]. CLL is also known to be correlated with an increase in secondary malignancies. CLL is also known to be correlated to an increase in secondary neoplasms. In addition to causative factors such as previous exposure to chemotherapeutic agents, genetic susceptibility, and other factors such as viral infections or smoking, secondary neoplasms are also correlated to the state of immunosuppression typical of this disease; studies indicated the state of immunosuppression of previous neoplasia as potential CLL causative factors [3].

Several polymorphisms are related to the p53 pathway; codon 72 and intron 6 appear to have a more significant impact on cancer risk [4]. Codon 72 is present in exon 4 of *TP53* within a proline-rich domain, which lies between the N-terminal transactivating domain and the DNA-binding domain which harbors most of the tumor-associated mutations [5]. This polymorphism yields two variants of the protein, one with an arginine residue (CGC) and another with proline (CCC).

Comparative sequence analyses in non-human primates suggest that p53-P72 is the wild type, although p53-R72 occurs at a high frequency (>50%) in some populations [6]. This polymorphism's ethnic and geographical distribution varies. The p53-P72 allele frequency is ~60% in the African population and ~30% in the Caucasian population [7]. The p53-P72 allele shows weaker activity in inducing apoptosis and suppressing cellular transformation [8]. It also shows lower transcriptional activity toward a subset of p53 target genes involved in apoptosis and DNA repair than the p53-R72 allele [9]. *TP53* codon 72 polymorphism reportedly leads to greater longevity and survival after a cancer diagnosis or other life-threatening diseases, but does not decrease cancer risk [10].

The relationship of *TP53* codon 72 polymorphisms with the risk of cancer and survival has been reported [5]; however, these survival studies were affected by problems similar to those described for cancer risk association studies. Moreover, none of the extensive studies with statistically robust results have reported significant associations to date [11]; even a meta-analysis found no relationship between codon 72 and hematologic malignancies but indicated that the number of included studies was very limited [12].

Similarly, the relationship of *TP53* codon 72 polymorphisms in CLL has been previously studied; but no reliable results have, yet, been obtained [13-15].

The intron 6 polymorphism transition from G to A is located after 61 nucleotides from exon 6 and abrogates a restriction endonuclease site MspI (CCGG to CCAG). It is associated with some types of cancers [16, 17]. However, this polymorphism is not related to CLL development risk, or a worse prognosis [14].

Both codon 72 and intron 6 polymorphisms of *TP53* have been studied in many cancer types, yielding varied results. In CLL, the results have been generally inconclusive. Therefore, this study aimed to analyze the relationship of the genotypes of polymorphisms of codon 72 and intron 6 of TP53 in a large cohort of patients with CLL.

PATIENTS AND METHODS

Chronic lymphocytic leukemia patients

We included and analyzed 558 consecutive Spanish patients diagnosed with CLL; all were referred from a group of associated hospitals (Hospital Universitario Puerta de Hierro-Majadahonda, Hospital Universitario de Getafe, and Hospital Universitario Severo-Ochoa) for the diagnostic evaluation of chronic lymphoproliferative disorders. All were followed up to determine progression, treatment, and survival. This study was approved by the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda and was conducted per the recommendations of the Declaration of Helsinki. All patients provided written informed consent for the use of their blood samples and the clinical data

of their disease evolution in the present study. All patients were Spanish Caucasian individuals.

CLL diagnosis was based on clinical characteristics, the presence of B-lymphocytes ≥ 5.0 x 10^9/L, blood morphology, and immunophenotype [18]. Moreover, to support the immunophenotyping study, the expression of CD38 and ZAP-70 was determined wherever possible [18]. Molecular cytogenetic characterization was performed prospectively. Karyotype analysis, fluorescence *in situ* hybridization (FISH) analysis with probes for 13q14(D13S25), 11q22-23, chromosome 12, 17p13.1 [19], and immunoglobulin heavy chain variable region gene (IgHV) sequencing were performed in all cases as previously described [18]. A germline homology of 98% was used as the cut-off point between mutated and non-mutated IgHV cases [20]. *TP53* sequencing was performed using the Sanger method, which has been previously described [21]; depending on the time of diagnosis of each patient, the European Research Initiative on CLL (ERIC) recommendations were followed [21]. Furthermore, wherever applicable, retrospective mutational screening for a NOTCH1 panel was performed using previously described techniques [19].

The clinical stage was determined using the Binet staging system [22], and CLL treatment was based on the International Workshop requirements on CLL (IWCLL) recommendations [18]. This cohort, which had a very long follow-up period, constituted patients receiving treatment regimens based on chemotherapy, immunochemotherapy, and targeted therapy. Refractory disease was defined as treatment failure or as progression within 6 months from the last dose of therapy with purine analogs +/- monoclonal antibodies or targeted therapy [18, 23].

Analysis of TP53 codon 72 and intron 6 polymorphism

Per the recommended procedure, genomic DNA was extracted from peripheral lymphocyte and mononuclear cell preparations from patients at diagnosis using standard methods. The polymorphisms were prospectively determined using real-time polymerase chain reaction (RT-PCR) with the Light Cycler® 2.0 RT-PCR System from Roche Life Science™, using primers described above and minimally modified conditions [7, 14, 16, 17, 24, 25].

For codon 72, the following primers were used: sense (S) 5´-LC640-TCCCCCCgTIICCCCTgCACC-PH, and antisense (A) 5´-CCAgATgAAgCTCCCAgAATgCCAgAggCT-FL.

For intron 6 the following primers were used: (S) 5´-CCCTCCAgTAggTg — FL, and (A) LC640-gggCTTTCTCCTgCTgCTTATTTgACC — PH.

In many of the cases, codon 72 of TP53 was determined prospectively through direct sequencing and identifying the same polymorphisms during diagnosis and subsequent relapses.

Statistical analysis

A descriptive analysis of all the included variables was performed. Absolute and relative frequencies were used to describe qualitative variables; quantitative variables were described using a central tendency measure (mean or median) accompanied by their dispersion measure (standard deviation or interquartile range) according to the variable's nature. Intergroup comparisons were conducted using the χ^2 test (or Fisher's exact test when necessary) for qualitative variables and the Student's T-test or analysis of variance (ANOVA) for quantitative variables with a normal distribution, or the Mann–Whitney U test or Kruskal–Wallis test for quantitative variables with a non-normal distribution. Survival curves were obtained using the Kaplan–Meier method, and comparisons between different groups were performed with the log-rank test. Multivariate analysis was subsequently performed using Cox regression. Hazard ratios (HRs) of the dependent variables were estimated along with their 95% confidence intervals (95% CIs). A p-value < 0.05 was considered statistically significant. All calculations were performed using the statistical program SPSS® v18.

RESULTS

Patient characteristics

The patient database included clinical data, immunophenotyping results, FISH data, and IgHV and karyotype data at the time of diagnosis and during follow-up for 558 patients with a CLL diagnosis, of which 58.8% (n = 328) were male

and 41.2% (n = 230) were female. During diagnosis, 30 patients (5%) with monoclonal lymphocytosis were included, who subsequently progressed to CLL. The median patient age was 63.3 years (range, 29-99 years). At the time of diagnosis, in assessments using the Binet staging system, 75.9%, 20.5%, and 3.6% of the patients had stage A, stage B, and stage C disease, respectively. The median follow-up period was 122.5 months (interquartile range: 73.4-180.4 months). Of these patients, 8.7% (48 patients) showed RT.

A total of 348 patients were treated with a median of 2.4 treatment regimens. Over the extended follow-up period, many patients only received conventional chemotherapy, while others received immunochemotherapy or targeted therapy. In this cohort, 39 patients underwent bone marrow transplantation (autologous transplantation, 10 patients; allogeneic transplantation, 29 patients). A total of 52 patients were classified as showing refractory disease.

Follow-up assessments showed secondary neoplasms in 19.7% (n = 110) of the patients, with the tumors appearing in the bladder, skin, and colon (14.5% each), prostate (13.6%), and the lung (12.7%). During the study period, 335 patients died. The descriptive clinical data are provided in Table 1.

TP53 codon 72 polymorphisms

There were 321 patients with Arg/Arg, 202 with Arg/Pro, and 35 with Pro/Pro. After univariate analysis, sex and age distribution for all genotypes of codon 72 polymorphism were similar. In a comparative analysis of the three groups, the Pro/Pro genotype was significantly associated with advanced Binet stage B and C disease, and showed a higher number of advanced cases than the other genotypes (p = 0.002).

Patients with the Pro/Pro genotype also showed a higher incidence of RT with a significant association (p = 0.013), and a significant association (p = 0.030) with the time to first treatment (TTFT), while patients with the Arg/Pro genotype showed a longer TTFT than those with the other genotypes. The median overall survival (OS) was 156.32 months (139.92-172.72 months), showing that patients with the Arg/Pro genotype live significantly longer (40 months) than the other groups (p = 0.028) (see Figure 1).

Secondary neoplasms appeared in 19.7% (110/558) of the patients, and their appearance was significantly associated with the homozygous genotypes (Arg/Arg and Pro/Pro) than with the Arg/Pro genotype, which on the contrary, curiously presented fewer secondary neoplasms (p = 0.016) (see Table 2). No associations were found between codon 72 and CD38+, ZAP70+, complex karyotype (CK), IgHV, NOTCH1, del(11q), +12, del(17p), del(13q), and the TP53 mutation. Finally, in the multivariate analysis, age, CK, del(11q), del(17p), non-mutated IgHV, and Pro/Pro genotype at codon 72 were identified as independent variables associated with an increased risk of death (see Table 3).

TP53 intron 6 polymorphisms

We analyzed intron 6 polymorphisms in 444 patients (G/G, n = 331; G/A, n = 101; and A/A, n = 12). It showed no significant associations with clinical characteristics, but A/A genotype group contained a substantially greater proportion of patients refractory to treatment (16.7%) and with secondary neoplasms (25%).

The A/A genotype was significantly correlated (p = 0.036) with del(11q) (33.3% of the patients). Moreover, although this group also showed a higher rate of del(17p) (50%), the association was not significant. Survival analysis showed a shorter median OS in patients with A/A genotype; however, this difference was not significant.

DISCUSSION

The findings of this study suggest that the presentation of specific genotypes of codon 72 of *TP53* in patients with CLL have prognostic significance. The Pro/Pro genotype was associated with a higher rate of RT and shortened TTFT in comparison with other genotypes. On the other hand, the Arg/Pro genotype was associated with a lower incidence of RT and a longer TTFT. Moreover, a higher incidence of secondary neoplasms was observed in the homozygous groups (Arg/Arg and Pro/Pro) than in the heterozygous group (Arg/Pro), and overall survival was longer in carriers of the Arg/Pro genotype. The Pro/Pro genotype was associated

Table 1. Characteristics of the study population.

		Global ([1]n = 558) — No. of patients (%)
Age (Years) — Median ([2]SD); Range = 63.3 (11.7); 29-99		
Distribution	>65 years	251 (46.1)
	≤65 years	294 (53.9)
Sex	Male	328 (58.8)
	Female	230 (41.2)
Binet stage	A	419 (75.9)
	B	113 (20.5)
	C	20 (3.6)
[3]LDT (n = 475)	<12 months	97 (20.4)
	>12 months	378 (79.6)
CD38 (n = 491)	Positive	87 (17.7)
ZAP-70 (n = 261)	Positive	109 (41.8)
Karyotype (n = 518)	Complex	88 (17)
del(17p)	Positive	82 (14.7)
TP53 mutation status (n = 191)	Mutated	55 (28.8)
del(11q)	Positive	53 (10.0)
IgHV (n = 494)	Mutated	259 (52.4)
	Unmutated	235 (47.6)
NOTCH1 (n = 190)	Mutated	35 (18.4)
Richter transformation		48 (8.7)
[4]TTFT (months) — Median (SD); Range = 48.2 (51.2); 0.07-268.6		
Type of treatment (n = 348)	Chemotherapy	140 (40.2)
	Immuno-chemotherapy	151 (43.4)
	Direct	57 (16.4)
Refractory to treatment		52 (9.3)
Secondary neoplasm		110 (19.7)
Mortality		335 (60)

[1]n: number of patients; [2]SD: Standard deviation; [3]LDT: Lymphocyte doubling time; [4]TTFT: Time to first treatment.

with an independent risk of death and was significantly similar to other risk factors such as advanced age, CK, del(11q), del(17p), non-mutated IgHV genes, and advanced Binet stage. However, while the A/A genotype of intron 6 of TP53 showed a relationship with del(11q), it was not associated with significantly increased mortality.

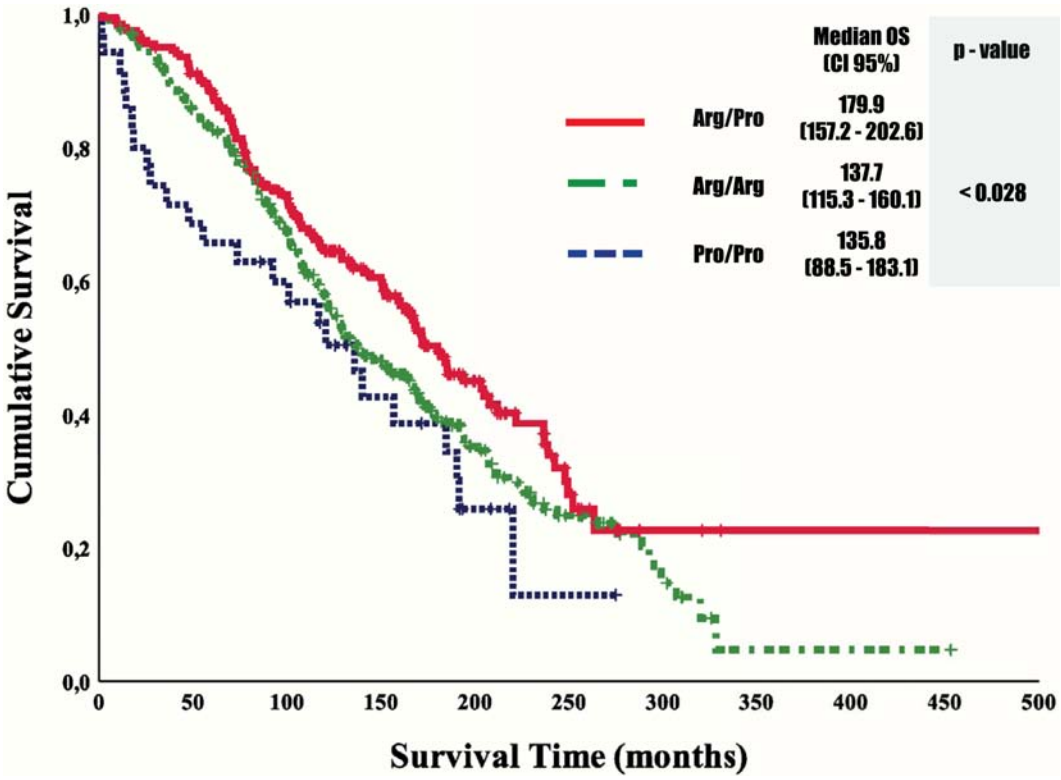

Figure 1. Overall survival of the codon 72 TP53 genotype groups.

Table 2. Clinical and biological characteristics according to TP53 codon 72 genotypes.

		Global [1]n = 558 (%)	Codon 72			[2]*p*-value
			Arg/Arg n = 321 (57.5)	Arg/Pro n = 202 (36.2)	Pro/Pro n = 35 (6.3)	
[3]HWE — No. of patients (%)		558	319 (57.2)	206 (36.9)	33 (5.9)	0.181
Binet stage — No. of patients (%)	**A**	419 (75.9)	242 (76.6)	157 (78.1)	20 (57.1)	**0.002**
	B	113 (20.5)	65 (20.6)	39 (19.4)	9 (25.7)	
	C	20 (3.6)	9 (2.8)	5 (2.5)	6 (17.1)	
Richter transformation — No. of patients (%)		48 (8.7)	33 (10.4)	9 (4.5)	6 (17.1)	**0.013**
[4]TTFT (Months) — Median ([5]SD); range		48.2 (51.2); 0.07-268.6	51.1 (54.2)	48.9 (47.9)	21.9 (34)	**0.030**
Secondary neoplasm — No. of patients (%)		110 (19.7)	74 (23.1)	27 (13.4)	9 (25.7)	**0.016**
Mortality — No. of patients (%)		335 (60)	203 (63.2)	108 (53.5)	24 (68.6)	**0.048**

[1]n: number of patients; [2]p: χ^2 test or Student's test; [3]HWE: Hardy – Weinberg Equilibrium; [4]TTFT: Time to first treatment; [5]SD: Standard deviation.

Table 3. Multivariate analysis.

		[1]HR ([2]95%CI)	[3]*p*-value
Age (>65 years versus <65 years)		3.267 (2.521 - 4.233)	**<0.001**
Karyotype (Complex versus No complex)		1.718 (1.220 - 2.421)	**0.002**
del(11q)		1.625 (1.134 - 2.328)	**0.008**
del(17p)		1.737 (1.221 - 2.471)	**0.002**
IgHV (Unmutated versus Mutated)		2.105 (1.599 - 2.769)	**<0.001**
Binet (B+C versus A)		1.914 (1.445 - 2.533)	**<0.001**
Codon 72 TP53	**Arg/Pro**	*Reference*	-
	Arg/Arg	1.261 (0.970 - 1.640)	0.084
	Pro/Pro	1.728 (1.062 - 2.810)	**0.028**

[1]HR: Hazard ratio; [2]95% CI: Confidence interval; [3]p = Cox regression.

Our cohort contained a significantly greater number of patients with the Arg/Arg genotype and fewer patients with the Pro/Pro genotype, similar to the proportions reported in a study of healthy patients in Europe [7] and in other studies on patients with CLL also from Europe [13-15, 26, 27]. However, this frequency differed from the findings for a Chinese cohort with CLL, in which Arg/Pro was the most frequent genotype and Pro/Pro also showed a lower incidence, but the incidence of Pro/Pro was higher than that in other European studies [28].

We did not compare our cohort with a healthy population, as opposed to a study that did and found that the Arg/Arg genotype was significantly related to the tendency to develop CLL [29]. Polymorphisms of codon 72 have been previously studied in CLL, although these studies yielded mixed results and did not show solid associations relating these polymorphisms to a worse disease evolution. In other studies, age [13, 14, 28] and sex [13, 28] were not associated with polymorphisms of codon 72.

In contrast to other publications [13-15, 28] that reported no significant associations with the Binet stage, our study showed a meaningful relationship between the Pro/Pro genotype and advanced Binet stages B and C. Another previous study that analyzed codon 72 in a subgroup of patients with *TP53* mutations also showed an association with Binet stage B [27]. One study also showed significant association between CD38 negativity and the Arg/Arg genotype [14]; however, like other studies [13, 28], we did not find a significant relationship between CD38 negativity and codon 72 polymorphisms. Likewise, ZAP-70 did not show an association with codon 72 polymorphisms in our study, similar to two other studies [13, 28].

In a previous study [14], the CK did not show an association with codon 72 polymorphisms. However, in our study, the percentage of patients with the Pro/Pro genotype and CK was higher than the rest. Nevertheless, this association was not significant. The del(17p) has been shown to be related to the Pro/Pro genotype [28]; however, in our cohort, although the percentage of patients with the Pro/Pro genotype with del(17p) was high, no significant associations were found, similar to a previous study [26].

We did not find a significant association with *TP53* mutations in our study, in contrast to three studies that reported significant correlations, with two showing correlations with the Pro/Pro genotype [27, 30] and the third reporting a significant correlation with the Pro72 allele (fusion of genotypes Arg/Pro and Pro/Pro) [28]. The del(11q) was not associated with codon 72 genotypes in our study. In a subgroup of CLL patients with unmutated IgHV, a significant association was observed between del(11q) and

the Pro/Pro genotype of *TP53* [31]. However, the mutational status of IgHV also did not show statistical significance in our study, similar to other reviews [13-15, 27, 31].

On the other hand, however, one study found significant differences in the frequency of codon 72 TP53 genotypes in relation to IgHV status [26]. The Arg/Arg genotype was found in a greater number of patients with unmutated CLL IgHV, and the Pro/Pro genotype was mainly in patients with mutated CLL IgHV [26].

Unlike previous studies, we did evaluate the association between NOTCH1 and polymorphisms of codon 72 of TP53; however, we did not find any association with genotypes, perhaps because we did not carry out the study in all patients with codon 72 polymorphisms. In our cohort, patients with the Pro/Pro genotype had a shorter TTFT, similar to other studies [26, 31], and this characteristic was independent of IgHV mutational status. In contrast, the Arg/Pro genotype showed a significant association with a longer TTFT, despite the presence of the Pro allele.

In our study, although a considerably greater number of chemorefractory patients were observed in the Pro/Pro group, this trend was not significant, unlike another study that reported a significant association in a subgroup of patients with mutated *TP53*, where the allele Pro72 (Arg/Pro and Pro/Pro) showed an association with chemorefractory status [28].

Research in gastric cancer has also reported a correlation between codon 72 Pro/Arg and Pro/Pro genotypes and a lower response rate to chemotherapy [32]. Notably, our study reported an association between the homozygous Arg/Arg and Pro/Pro genotypes at codon 72 with a higher incidence of secondary neoplasms, which most frequently manifested as neoplasms of the bladder, skin, colon, prostate, and lung. There are numerous evidences that patients with solid tumors who had a Pro/Pro genotype had a worse outcome and shorter survival [33-37], although studies have been published linking the Arg/Pro genotype and the Pro allele (Pro/Pro or Arg/Pro) with a worse prognosis [11, 38].

The present study observed a surprisingly statistically significant trend for longer survival in patients with the Arg/Pro genotype than in those with the Arg/Arg and Pro/Pro genotypes. This finding was also replicated in another study in which the Pro/Pro genotype was also shown to be associated with a shorter OS than Arg/Arg [28]. We confirmed this association as an independent variable associated with a higher risk of death in the multivariate analysis. In contrast, in another study, a subgroup of CLL patients with a TP53 mutation carrying a Pro allele (Arg/Pro and Pro/Pro) at codon 72 of TP53 showed worse OS than patients with the same characteristics but with the Arg/Arg genotype for codon 72 [28]. Other studies reported no association of OS with codon 72 genotypes [14, 15, 30, 31].

Regarding intron 6 of *TP53*, a previous study of patients with CLL demonstrated an association with CD38+ status and TTFT; however, these findings did not show robustness in the multivariate analysis [14], and in another study, the intron was associated with a significant susceptibility to lung cancer [16]. Our cohort showed a significant association of the A/A genotype with del(11q); however, in the overall and multivariate survival analyses, we did not find associations with a higher risk of mortality in comparison with the other genotypes. We also found no associations between CD38 and TTFT.

The Pro/Pro genotype of *TP53* in patients with CLL represents a more aggressive presentation at diagnosis, and its characteristics have not been described in any other study on patients with CLL. The association of RT with the Pro/Pro genotype could be a genetic risk factor that predisposes CLL patients to a tumoral transformation. This association is significant since it has not been studied or demonstrated before and indicates the need for close follow-up of CLL patients, because early recognition of RT may facilitate a more targeted treatment. The presence of the Pro/Pro genotype also indicated a shorter TTFT, which was only demonstrated in other studies when associated with different biological alterations of the Pro/Pro genotype [26, 31]. These findings indicate the need for close follow-up of patients carrying this genotype.

The development of secondary tumors in patients with CLL has been previously reported to be associated with various factors. Nevertheless,

this is the first study to report the association of secondary tumor development with polymorphisms of codon 72 of *TP53*. The survival of patients with the homozygous genotypes at codon 72 of *TP53* (Arg/Arg and Pro/Pro) was affected by the development of secondary tumors. Therefore, these patients should be carefully monitored to promptly identify secondary tumors.

The presence of the Pro/Pro genotype has been shown to decrease overall survival and increase the risk of mortality. This result is consistent with another study in CLL patients carrying the same genotype [26].

The current study has some limitations. First, we did not compare the study group with a healthy population. Second, there was not enough DNA sample from all patients to study the genetic abnormalities of SF3B1, MYD88, and BIRC3; although we included patients with NOTCH1 data, the sample was not very large. Third, due to the extended follow-up of this cohort, some patients were lost.

CONCLUSION

Thus, we conclude that the Pro/Pro genotype of *TP53* codon 72 has a potential role in the progression and mortality of CLL patients. In contrast, the Arg/Pro genotype was associated with a lower risk of secondary neoplasms than the homozygous genotypes. Although our findings provided no evidence for significant associations of alterations of intron 6 with tumor-related characteristics, these alterations may be related to a worse prognosis and lower survival in CLL. Therefore, *TP53* polymorphisms and cancer risk are essential research areas requiring more attention and follow-up for more precise treatment.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Hallek, M. 2019, Am. J. Hematol., 94, 1266-1287.
2. Rossi, D. and Gaidano, G. 2016, Semin. Oncol., 43, 311-319.
3. Royle, J. A., Baade, P. D., Joske, D., Girschik, J. and Fritschi, L. 2011, Br. J. Can., 105, 1076-1081.
4. Pietsch, E. C., Humbey, O. and Murphy, M. E. 2006, Oncogene, 25, 1602-1611.
5. Whibley, C., Pharoah, P. D. P. and Hollstein, M. 2009, Nat. Rev. Can., 9, 95-107.
6. Puente, X. S., Velasco, G., Gutiérrez-Fernández, A., Bertranpetit, J., King, M. C. and López-Otín, C. 2006, BMC Genomics, 7, 1-9.
7. Själander, A., Birgander, R., Saha, N., Beckman, L. and Beckman, G. 1996, Hum. Hered., 46, 41-48.
8. Dumont, P., Leu, J. I., Della Pietra, A. C., George, D. L. and Murphy, M. 2003, Nat. Genet., 33, 357-365.
9. Jeong, B-S., Hu, W., Belyi, V., Rabadan, R. and Levine, A. J. 2009, FASEB J., 24, 1347-1353.
10. Ørsted, D. D., Bojesen, S. E., Tybjærg-Hansen, A. and Nordestgaard, B. G. 2007, J. Exp. Med., 204, 1295-1301.
11. Matakidou, A., el Galta, R., Webb, E. L., Rudd, M. F., Bridle, H., Eisen, T. and Houlston, R. S. 2007, Lung Cancer, 57, 207-212.
12. Weng, Y., Lu, L., Yuan, G., Guo, J., Zhang, Z., Xie, X. and Chen, G. 2012, PLoS One, 7, e45820.
13. Lahiri, O., Harris, S., Packham, G. and Howell, M. 2007, Can. Genet. Cytogenet., 179, 36-44.
14. Kochethu, G., Delgado, J., Pepper, C., Starczynski, J., Hooper, L., Krishnan, S., Fegan, C. and Pratt, G. 2006, Leuk. Res., 30, 1113-1118.

15. Sturm, I., Bosanquet, A. G., Hummel, M., Dörken, B. and Daniel, P. T. 2005, BMC Cancer, 5, 1-5.
16. Biroš, E., Kalina, I., Kohút, A., Štubňa, J. and Šalagovič, J. 2001, Lung Cancer, 31, 157-162.
17. Mavridou, D., Gornall, R., Campbell, I. G. and Eccles, D. M. 1998, Br. J. Can., 77, 676-677.
18. Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., Hillmen, P., Keating, M., Montserrat, E., Chiorazzi, N., Stilgenbauer, S., Rai, K. R., Byrd, J. C., Eichhorst, B., O'Brien, S., Robak, T., Seymour, J. F. and Kipps, T. J. 2018, Blood., 131, 2745-2760.
19. Lozano-Santos, C., García-Vela, J. A., Pérez-Sanz, N., Nova-Gurumeta, S., Fernandez-Cuevas, B., Gomez-Lozano, N., Sánchez-Beato, M., Sanchez-Godoy, P., Bueno, J. L. and Garcia-Marco, J. A. 2017, Leuk. Lymphoma, 58, 859-865.
20. Rosenquist, R., Rosenwald, A., Du, M. Q., Gaidano, G., Groenen, P., Wotherspoon, A., Ghia, P., Gaulard, P., Campo, E. and Stamatopoulos, K. 2017, Leukemia, 31, 1477-1481.
21. Malcikova, J., Smardova, J., Pekova, S., Cejkova, S., Kotaskova, J., Tichy, B., Francova, H., Doubek, M., Brychtova, Y., Janek, D., Pospisilova, S., Mayer, J., Dvorakova, D. and Trbusek, M. 2008, Mol. Inmunol., 45, 1525-1529.
22. Binet, J. L., Auquier, A., Dighiero, G., Chastang, C., Piguet, H., Goasguen, J., Vaugier, G., Potron, G., Colona, P., Oberling, F., Thomas, M., Tchernia, G., Jacquillat, C., Boivin, P., Lesty, C., Duault, M. T., Monconduit, M., Belabbes, S. and Gremy, F. 1981, Cancer, 48, 198-206.
23. Reyes, C., Gauthier, G., Shi, S. and Guerin, A. 2018, J. Can. Ther., 09, 576-587.
24. Peller, S., Kopilova, Y., Slutzki, S., Halevy, A., Kvitko, K. and Rotter, V. 1995, DNA Cell Biol., 14, 983-990.
25. Chumakov, P. M. and Jenkins, J. R. 1991, Nucleic Acids Res., 19, 6969.
26. Majid, A., Richards, T., Dusanjh, P., Kennedy, D. B. J., Miall, F., Gesk, S., Siebert, R., Wagner, S. D. and Dyer, M. J. S. 2011, Br. J. Haematol., 153, 533-535.
27. Bilous, N. I., Abramenko, I. V., Chumak, A. A., Dyagil, I. S. and Martina, Z. V. 2014, Exp. Oncol., 36, 258-261.
28. Dong, H. J., Fang, C., Wang, L., Fan, L., Xu, J., Wu, J-Z., Lu, T-X., Li, J-Y. and Xu, W. 2014, Med. Oncol., 31, 908.
29. Biroš, E., Kalina, I., Biroš, I., Kohút, A., Bogyiová, E., Šalagovič, J. and Štubňa, J. 2001, Neoplasma., 48, 407-411.
30. Grossmann, V., Artusi, V., Schnittger, S., Dicker, F., Jeromin, S., Boeck, L., Haferlach, T., Haferlach, C., Kern, W. and Kohlmann, A. 2011, Blood, 118, 1783.
31. Lee, H., Liew, A., Lee, A., Haque, S., Kolitz, J. E., Allen, S. L., Rai, K. R., Gregersen, P., Chu, C. C., Chiorazzi, N. and Mongini, P. 2011, Blood, 118, 3888.
32. Zha, Y., Gan, P., Liu, Q. and Yao, Q. 2016, Arch. Med. Res., 47, 13-18.
33. Tommiska, J., Eerola, H., Heinonen, M., Salonen, L., Kaare, M., Tallila, J., Ristimäki, A., Von Smitten, K., Aittomäki, K., Heikkilä, P., Blomqvist, C. and Nevanlinna, H. 2005, Clin. Can. Res., 11, 5098-5103.
34. Xu, Y., Yao, L., Ouyang, T., Li, J., Wang, T., Fan, Z., Lin, B., Lu, Y. and Xie, Y. 2005, Clin. Can. Res., 11, 7328-7333.
35. Wang, Y-C., Chen, S-K., Lee, H-S. and Chen, C-Y. 1999, Clin. Can. Res., 5, 129-134.
36. Almquist, L. M., Karagas, M. R., Christensen, B. C., Welsh, M. M., Perry, A. E., Storm, C. A. and Nelson, H. H. 2011, Carcinogenesis, 32, 327-330.
37. Hori, Y., Miyabe, K., Yoshida, M., Nakazawa, T., Hayashi, K., Naitoh, I., Shimizu, S., Kondo, H., Nishi, Y., Umemura, S., Kato, A., Ohara, H., Inagaki, H. and Joh, T. 2015, PLoS One, 10, 1-13.
38. Rivu, S. F., Apu, M. N. H., Shabnaz, S., Nahid, N. A., Islam, M. R., Al-Mamun, M. M. A., Nahar, Z., Rabbi, S. N. I., Ahmed, M. U., Islam, M. S. and Hasnat, A. 2017, Can. Epidemiol., 49, 46-52.