Paraoxonase 1 (PON1) Q192R and L55M Polymorphisms as Potential Predisposition Factors for Chronic Lymphocytic Leukemia

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Abstract. *Background/Aim:* PON1 gene has an executive role in antioxidant defense, protecting cells from genotoxic factors. Q192R and L55M PON1 polymorphisms reduce catalytic activity of the encoded protein. These polymorphisms were studied in 300 chronic lymphocytic leukemia (CLL) patients and 106 healthy donors. They were also associated with patients' cytogenetic findings, to investigate their possible implication in CLL pathogenesis. *Materials and Methods:* SNP genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Karyotypic analysis was also performed by chromosome G-banding analysis and fluorescence in situ hybridization. *Results:* Genotypic and allelic distribution of Q192R polymorphism showed a statistically significant higher frequency of mutant genotypes and mutant alleles in patients compared to controls. The same observation was noted in patients with abnormal karyotypes and those carrying *abn14q32* and *del(6q)*. A statistically increased frequency for the mutant allele was also revealed in patients with *del(11q)*. On the contrary, L55M polymorphism showed a similar distribution between patients and controls. *Conclusion:* Q192R polymorphism plays a role in CLL predisposition and the formation of specific chromosomal aberrations.

Chronic lymphocytic leukemia (CLL), a clonal neoplasia of B-lymphocytes and the most common type of adult leukemia

in the Western world is a disease of the elderly. The median age at diagnosis is 72 years (1, 2). CLL is characterized by the presence of recurrent cytogenetic abnormalities including *del(13q)*, trisomy 12, *del(11q)*, *del(6q)*, *del(17p)* and *t/der(14)(q32)*, which are important diagnostic and prognostic factors and contribute to the pathogenesis of the disease (3). Deletion of 13q appears to be the most frequent cytogenetic abnormality, while deletion or rearrangement of 17p, a less frequent abnormality resulting in loss or mutation of *TP53* gene confers the worst survival in CLL (4-7).

Although CLL pathogenesis has not been yet fully elucidated, epidemiological studies support an important role of genetics, immune function, infections and variable environmental factors in CLL pathogenesis (8, 9). Indeed, CLL appears to show a relatively common familial aggregation supporting genetic susceptibility and marks heritability of the disease as a significant risk factor (10-13).

Recently, it has been postulated that CLL results from a combination of genotoxic exposure influenced by genetic susceptibility. Since CLL is a leukemia of advanced age, it is unlikely that genetic susceptibility to CLL relies mainly on mutations in crucial genes such as tumor suppressor genes which would result in the onset of the disease in younger ages. Therefore, its onset could be possibly attributed to the accumulation of toxic compounds in lymphoid or other interacting tissues as a result of chronic exposure to genotoxic agents due to ineffective detoxification (14).

The risk of CLL has already been associated with polymorphic variants of genes encoding enzymes involved in the enzyme activation and detoxification processes (15-17). Human serum paraoxonases (PONs) are enzymes that play an executive role in antioxidant defense mechanisms, protecting cells from genotoxic factors like organophosphates and the accumulation of reactive oxygen species (ROS) (*e.g.* peroxides, superoxide, hydroxyl radical, and singlet oxygen).

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They also modulate oxidative stress (OS) and inflammation, factors that mediate carcinogenesis by causing metabolic malfunction and DNA damage (18, 19). In addition, it has been proposed that altered PON enzymatic activity could be involved in tumor survival and stress resistance.

PON1, a member of PONs, exhibits lactonase and ester hydrolase activity and was primarily associated with plasma high-density lipoprotein (HDL). However, it appears to oxidize lipids and hydrolyze active metabolites of several organophosphorus pesticides protecting cells by playing a vital role in antioxidant defense (20). Moreover, a lower PON1 serum activity and overexpression of the other PONs, PON2 and PON3 genes have been reported in cancer patients. *PON1* gene, located on 7q21.3 chromosome region, is subjected to two single nucleotide polymorphisms (SNPs) in its coding region that result in amino acid substitutions at positions 55 and 192, influencing the stability, concentration and enzyme catalytic activity. More specifically, L55M polymorphism (rs854560) results in leucine to methionine substitution in position 55 and Q192R polymorphism (rs662) results in glutamine to arginine substitution at position 192 of the encoded protein product (21, 22). It is worth noting that Q192R and L55M *PON1* polymorphisms have been shown to account for more than 60% of the inter-individual variations in enzyme concentration and activity (23) and in many studies they have been associated with several human cancers, but their possible association with CLL has been reported recently only in a small group of patients (19, 24-27).

In the current study, Q192R and L55M *PON1* polymorphisms were examined in a large group of CLL patients in order to investigate their possible implication in CLL pathogenesis and in the formation of specific CLL chromosomal aberrations.

Materials and Methods

Study population. Our study included 300 CLL patients selected based on their cytogenetic findings and 106 healthy individuals. The diagnosis of CLL was established according to the World Health Organization (WHO) classification and the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria (28, 29). Healthy donors were unrelated Greek individuals from the general population of Greece, with a negative history of previous malignancies and normal peripheral blood cell counts. The study was approved by the Ethics Committee of the University of Athens and the Scientific Committee of 'Sotiria' General Hospital of Athens (IRB number: 11462, 20/5/2015). Informed consent was obtained from all the enrolled individuals.

Chromosome G-banding analysis and Fluorescence in situ Hybridization (FISH). Karyotypic analysis was performed on unstimulated and stimulated with CpG-oligonucleotide DSP-30 (2 μ M; TIB Mol-biol, Berlin, Germany) and IL-2 (200 U/ml; Roche Diagnostics, Germany) bone marrow (BM)/peripheral blood (PB)

Figure 1. Partial G-banded karyotypes from two patients showing (A) *del(11)(q22q23)* and (B) trisomy 12.

cells in at least 20 metaphases and karyotypes were described according to ISCN 2016 (30). Complex karyotypes were defined as those with ≥ 3 clonal aberrations.

FISH was carried out using the commercial CLL set probes for the detection of the most common abnormalities of the disease including trisomy 12 (CEP 12) and deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.3/13q34.3 (D13S319/13q34) regions on at least 200 interphase cells (Vysis Inc., Downers Grove, IL, USA).

Genotype analysis. DNA isolation: Total genomic DNA was extracted from BM or PB of the enrolled CLL patients and healthy individuals using the QIAamp DNA-extraction midi kit (Qiagen, Hilden, Germany) following standard procedures according to the manufacturer's instructions. Extracted DNA was used as template for the subsequent genotypic analysis.

PON1 Q192R and L55M genotyping: The Q192R and L55M *PON1* SNPs genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. *PON1* alleles including locus 192 and locus 55 were separately amplified using the Q5 High-Fidelity DNA Polymerase (NEB, New England BioLabs) on the BIORAD CFX96 Thermocycler. For Q192R polymorphism, PCR reaction was performed using primers F: (5'TATTGTTGCTGTGGGACCTGAG3') and R: (5'CACGCTA AACCCTAAATACATCTC3') (31), following the conditions: 95°C for 30 sec followed by 40 cycles at 95°C for 30 sec, 57°C for 40 sec, 68°C for 1 min, and final extension at 68°C for 5 min. The amplified 99 bp PCR product was subsequently digested by Alw1 restriction endonuclease (NEB, New England BioLabs) and resulted in 99 bp, 69 bp and 30 bp fragments for the heterozygous genotype (QR), 69 bp and 30 bp for the homozygous mutant genotype (RR) and 99 bp non-digested fragment for the homozygous wild type genotype (QQ). For L55M polymorphism, PCR reaction was performed using primers F: (5'GAAGAGTGATGTATAGCCCCAG3') and R: (5'TTTAATCCAG AGCTAATGAAAGCC3') (31), following the conditions: 95°C for 30 sec followed by 35 cycles at 95°C for 30 sec, 55°C for 60 sec, 68°C for 30 sec, and final extension at 68°C for 5 min. The amplified 170 bp PCR product was digested by FAT1 restriction endonuclease (NEB, New England BioLabs) and resulted in 170 bp, 126 bp and 44 bp fragments for the heterozygous genotype (ML), 126 bp and 44 bp for the homozygous mutant genotype (MM) and a non-digested 170 bp fragment for the wild type genotype (LL). All PCR and digestion products were subjected to 2% agarose gel electrophoresis including negative and positive control samples.

Figure 2. FISH with CLL set of probes. (A) Metaphase FISH using LSI D13S319/LSI 13q34/CEP12 CLL set of probes showing trisomy 12. The red signal corresponds to the 13q14.3 region, the aqua signal to 13q34 region and the green signal corresponds to the centromere of chromosome 12. (B) Interphase and metaphase FISH using LSI p53/LSI ATM CLL set of probes demonstrating deletion of the 11q22.3 region. The red signal corresponds to the 17p13.1 region (p53), and the green signal corresponds to the 11q22.3 (ATM) region.

Statistical analysis. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 20 software. *PON1* genotypic and allele frequency distribution between CLL patients and healthy controls were compared by chi-square test. A p -value < 0.05 was considered significant. Odds ratios (ORs) were given with 95% confidence interval (CI). The Hardy-Weinberg equation was used to calculate the estimated and observed genotypic frequencies.

Results

Demographic characteristics. Sex ratio of CLL patients was 1.7 (187 males/113 females) vs. 1.3 (59 males/47 females) in the control group. The mean age was 65.3 years (range=35-94 years) for the patients vs. 54.9 years (range=30-92 years) for the control group. No significant differences were observed between patients and controls concerning mean age and sex ratio ($p > 0.05$).

Cytogenetic characteristics. Among the 300 selected CLL patients, 118 patients had normal karyotypes (also confirmed by normal FISH results) and 182 patients had abnormal karyotypes carrying at least one specific cytogenetic alteration. In particular, 61 patients carried del(13q), 56 trisomy 12, 40 del(11q), 24 del(17p), 21 patients abnormalities of 14q32 region [abn(14q32)], 12 abnormalities

of chromosome 8 [abn(8)], 9 patients del(6q) and 22 patients carried other chromosome abnormalities (Figures 1 and 2).

Among the 182 patients with abnormal karyotypes, 32 patients (17.6%) had numerical aberrations, 80 (44%) structural and 70 (38.4%) patients carried both numerical and structural aberrations. According to karyotypic complexity, 88 patients (48.3%) had a single aberration, 38 (20.9%) had two aberrations and 56 (30.8%) carried a complex karyotype (≥ 3 aberrations).

***PON1* Q192R SNP genotypic results.** The *PON1* Q192R SNP genotyping was successful in all 300 CLL patients and in 104 out of 106 healthy donors. In the control group, the *PON1* Q192R genotypes were distributed as follows: 63.5% wild type (QQ), 31.7% heterozygous (QR) and 4.8% homozygous for the mutant allele (RR). No deviation from the Hardy Weinberg equation was observed in the controls. Genotype distribution in CLL patients was: 48.3% wild type (QQ), 44% QR and 7.7% RR. Consequently, the genotypic distribution between CLL patients and healthy individuals was found to be significantly different, showing a higher frequency of the variant genotypes (heterozygotes QR and homozygotes RR) in patients compared to the controls (44% vs. 31.7% and 7.7% vs. 4.8% respectively) ($p = 0.028$, $\chi^2 = 7.14$, $df = 2$) (Table I). Moreover, CLL patients exhibited

Table I. Distribution of genotype and allele frequencies of the paraoxonase 1 (PON1) M/L55 and Q/R192 polymorphisms in CLL patients and healthy controls.

Group	rs854560 (L55M)					Group	rs662 (Q192R)				
	Genotype frequency			Allele frequency			Genotype frequency			Allele frequency	
	LL n (%)	ML n (%)	MM n (%)	L n (%)	M n (%)		QQ n (%)	QR n (%)	RR n (%)	Q n (%)	R n (%)
Healthy controls (n=105)	53 (50.5)	52 (49.5)	0 (0)	158 (75.2)	52 (24.8)	Healthy controls (n=104)	66 (63.46)	33 (31.73)	5 (4.81)	165 (79.33)	43 (20.67)
CLL patients (n=181)	91 (50.3)	89 (49.2)	1 (0.6)	271 (74.9)	91 (25.1)	CLL patients (n=300)	145 (48.33)	132 (44)	23 (7.67)	422 (70.33)	178 (29.67)
p-Value	p=0.747			p=0.834 OR (95% CI)=1.065 (0.814-1.326)		p-Value	p=0.028, x ² =7.14, df=2			p=0.012 OR (95% CI)=1.619 (1.030-1.776)	

OR: Odds ratio; CI: confidence interval.

a significantly increased frequency for the mutant allele (R) compared to controls (29.7% vs. 20.7% respectively) [*p*=0.012, OR (95%CI)=1.619(1.030-1.776)] (Table I).

Stratification of patients according to *Q192R* genotyping and karyotypic results showed that genotypic and allelic frequencies were comparable between patients with normal and abnormal karyotypes showing no significant correlation (Table II). Nevertheless, a statistically significant increased frequency for the mutant R allele was revealed in the subgroup of patients carrying *abn14q32* compared to the rest of the CLL patients (*p*=0.006). Additionally, comparison between each karyotypic subgroup of CLL patients and the control group revealed interesting correlations. More specifically, a statistical higher frequency of the variant genotypes as well as the variant alleles were observed in the group of patients with abnormal karyotypes compared to the control group (*p*=0.046 and *p*=0.019, respectively) (Table II). A similar result was also found in some specific karyotypic subgroups compared to the controls. In particular, the genotypic distribution results in patients carrying *abn14q32* and *del(6q)* were statistically significant compared to controls (*p*=0.030 and *p*=0.021 respectively), due to the increased incidence of heterozygotes (QR) and homozygotes for the mutant allele (RR) (Table II). Moreover, an increased frequency for the mutant allele was revealed in the CLL subgroup with *del(11q)* compared to the control group, reaching a statistically significant level (*p*=0.035).

Furthermore, when we compared patients with at least one mutant allele (QR+RR) with healthy controls some particular karyotypic subgroups reached a statistically significant level. More specifically, patients with normal karyotypes (*p*=0.023), as well as patients with abnormal karyotypes

(*p*=0.014) showed a statistically significant increased frequency of QR+RR genotypes compared to controls. A similar result was noticed in patients carrying *abn14q32* (*p*=0.011) and in those with *del(6q)* (*p*=0.015).

Further stratification of patients according to the type of chromosomal aberration (numerical/structural abnormalities) and the karyotype complexity (single aberration, two aberrations and complex karyotypes) along with *PON1 Q192R* genotype and allele frequencies missed the conventional threshold of significance.

PON1 L55M SNP genotypic results. This study was successful in all 181 CLL patients and in 105 out of 106 healthy donors. *PON1 L55M* SNP genotypic and allelic frequency distributions between CLL patients and healthy controls are also shown in Table I. The genotypic and allelic frequency distributions were similar between CLL patients and healthy controls. Stratification of CLL patients according to their cytogenetic findings and *PON1 L55M* SNP genotype results are shown in Table III. Genotype and allele frequencies between normal and abnormal karyotypes, and between each cytogenetic subgroup with the rest of the CLL patient groups, failed to reach statistical significance.

Further stratification of patients according to *PON1 L55M* SNP genotype results, the chromosomal aberration types (numerical vs. structural abnormalities) or the complexity of karyotype (single aberration, two aberrations and complex karyotypes) also failed to meet statistical significance.

Combined Q192R & L55M polymorphisms. In order to compare the results of both *PON1* gene polymorphisms, we concurrently examined both *PON1 Q192R* SNP and *PON1*

Table II. Stratification of CLL patients according to their cytogenetic findings, genotypic and allele frequencies of *PON1* Q192R polymorphism.

rs662 (Q192R)	Genotype					Allele				Genotypes with at least one mutant allele			
	n	QR	QQ	RR	<i>p</i> -Value	<i>p</i> -Value*	Q	R	<i>p</i> -Value	<i>p</i> -Value*	QR + RR	<i>p</i> -Value	<i>p</i> -Value*
		n (%)	n (%)	n (%)									
Controls	104	33 (31.73)	66 (63.5)	5 (4.81)			165 (79.33)	43 (20.67)			38 (36.5)		
Cytogenetic findings													
Normal Karyotype	118	52 (44.1)	57 (48.3)	9 (7.6)		0.075	166 (70.3)	70 (29.7)		0.030	61 (51.7)		0.023
Abnormal Karyotype	182	80 (43.9)	88 (48.4)	14 (7.7)	1.000	0.046	256 (70.3)	108 (29.7)	0.998	0.019	94 (51.7)	0.954	0.014
Trisomy 12	56	24 (42.9)	29 (51.8)	3 (5.4)	0.716	0.346	82 (73.2)	30 (26.9)	0.459	0.214	27 (48.2)	0.518	0.151
del(17p)/p53	24	8 (33.3)	14 (58.3)	2 (8.3)	0.541	0.763	36 (75)	12 (25)	0.461	0.511	10 (41.7)	0.307	0.640
del(13q)	61	23 (37.7)	32 (52.5)	6 (9.8)	0.489	0.266	87 (71.3)	35 (28.7)	0.791	0.098	29 (47.5)	0.470	0.165
del(11q)/ATM	40	14 (35)	20 (50)	6 (15)	0.060	0.086	54 (67.5)	26 (32.5)	0.551	0.035	20 (50)	0.710	0.140
abn(14q32)	21	13 (55.6)	7 (38.9)	1 (5.6)	0.229	0.030	72 (82.8)	15 (17.2)	0.006	0.499	14 (66.7)	0.154	0.011
del(6q)	9	7 (77.78)	2 (22.22)	0 (0)	0.109	0.021	11 (61.1)	7 (38.9)	0.425	0.074	7 (77.8)	0.111	0.015
abn(8)	12	4 (33.33)	6 (50)	2 (16.66)	0.438	0.243	16 (66.7)	8 (33.3)	0.688	0.156	6 (50)	0.906	0.363
Other abnormal Karyotypes	22	11(50)	11(50)	0 (0)	0.363	0.189	33 (75)	11 (25)	0.481	0.525	11 (50)	0.811	0.239

p-Value: Each group of patients with a specific chromosomal aberration was compared to the rest of the CLL patients. p-Value*: each group of patients carrying a specific chromosomal aberration was compared with healthy controls.

Table III. Stratification of CLL patients according to their cytogenetic findings, and genotypic and allele frequencies of *PON1* L55M polymorphism.

rs854560 (L55M)	Genotype				Allele				Genotypes with at least one mutant allele				
	n	ML	LL	MM	<i>p</i> -Value	<i>p</i> -Value*	L	M	<i>p</i> -Value	<i>p</i> -Value*	ML + MM	<i>p</i> -Value	<i>p</i> -Value*
		n (%)	n (%)	n (%)			n (%)	n (%)			n (%)		
Controls	105	53 (50.5)	52 (49.5)	0 (0)			158 (75.2)	52 (24.8)			53 (50.5)		
Cytogenetic findings													
Normal Karyotype	37	19 (51.4)	18 (48.6)	0 (0)		0.927*	55 (74.3)	19 (25.7)		0.876	19 (51.4)		0.927
Abnormal Karyotype	144	69 (47.9)	74 (51.4)	1 (0.7)	0.830	0.653	217 (75.7)	71 (24.7)	0.856	0.978	70 (48.6)	0.766	0.771
Trisomy 12	41	16 (39.02)	25 (60.98)	0 (0)	0.306	0.213*	66 (80.5)	16 (19.5)	0.203	0.340	16 (39.02)	0.139	0.253
del(17p)/p53	24	16 (66.7)	8 (33.3)	0 (0)	0.159	0.729*	32 (66.7)	16 (33.3)	0.145	0.224	16 (66.7)	0.066	0.129
del(13q)	54	26 (48.2)	27 (50)	1 (1.8)	0.306	0.371	80 (74.1)	28 (25.9)	0.760	0.821	27 (50)	0.884	0.955
del(11q)/ATM	38	18 (47.4)	20 (52.6)	0 (0)	0.856	0.743*	58 (76.3)	18 (23.7)	0.789	0.851	18 (47.4)	0.803	0.820
abn(14q32)	21	9 (42.9)	12 (57.1)	0 (0)	0.787	0.524*	33 (78.6)	9 (21.4)	0.584	0.645	9 (42.9)	0.538	0.577
del(6q)	9	3 (33.33)	6 (66.67)	0 (0)	0.552	0.324*	15 (83.3)	3 (16.7)	0.409	0.441	3 (33.3)	0.330	0.351
abn(8)	13	7 (53.8)	6 (46.2)	0 (0)	0.932	0.819*	19 (73.1)	7 (26.9)	0.801	0.810	7 (53.8)	0.726	0.769
Other abnormal Karyotypes	10	4 (40)	6 (60)	0 (0)	0.764	0.527*	16 (80)	4 (20)	0.605	0.635	4 (40)	0.515	0.565

p-Value: Each group of CLL patients with a specific chromosomal aberration was compared with the rest of the CLL patients. p-Value*: each group of CLL patients carrying a specific chromosomal aberration was compared with healthy controls.

Table IV. Stratification of CLL patients and controls according to the combination of both *PON1* L55M and Q192R genotypes.

PON1 polymorphisms Q192R\L55M	a. QQ\LL n (%)	b. QQ\ML+MM n (%)	c. QR+RR\LL n (%)	d. QR+RR\ML+MM n (%)	p-Value	e. (At least one mutation) n (%)	*p-Value (between column a and e)
CLL (n=180)	30 (16.7)	55 (30.6)	61 (33.9)	34 (18.9)	$p=0.006$, $\chi^2=12.5$, $df=3$	150 (83.3)	$p=0.003$, $\chi^2=8.95$, $df=1$
Controls (n=103)	33 (32.1)	32 (31.1)	19 (18.4)	19 (18.4)		70 (67.9)	

L55M SNP genotypic and allelic frequency distributions in the 180 CLL patients (for those we had results for both *Q192R* & *L55M* polymorphisms) and 103 healthy controls. These results are presented in Table IV. In the control group, the frequency of the wild type genotypes for both polymorphisms (QQ/LL) was higher (32.1%) compared to the CLL group (16.7%), indicating that the presence of at least one mutation is frequently observed in the CLL group compared to the controls ($\chi^2=8.95$, $df=1$, $p=0.003$).

The concurrent association of *Q192R* and *L55M* genotypic and allelic frequency distributions with the cytogenetic findings of CLL patients, showed comparable frequencies between patients with normal and abnormal karyotypes ($p=0.117$). A higher frequency of patients with at least one *PON1* mutation was found in the subgroup of patients with normal karyotype (91.9%) and in the subgroup of patients with *abn(14q32)* (86.4%) compared to the rest of the CLL patients (data not shown).

Finally, to evaluate how strong the *Q192R PON1* polymorphism is related to the CLL development, we calculated the odds ratio of having the disease and *PON* polymorphism versus the odds of having the disease without *PON* polymorphism by a 2x2 table divided by presence/absence of the disease and presence/absence of the selected polymorphism. The OR=1.85 indicates that CLL is more likely to occur with the *Q192R PON1* polymorphism. The corresponding study on *L55M* polymorphism did not reveal any relation.

Discussion

Several reports suggest that genetic predisposition along with exposure in environmental genotoxic compounds may play a pivotal role in the pathogenetic pathways implicated in CLL development (9-11, 16). In the current study we hypothesized that the accumulation of toxic compounds with the passage of years, as a result of non-effective detoxification, inefficient antioxidant defense mechanisms or defective DNA repair mechanism merely attributed to minor genetic changes such as, genetic variation in the human genome, may explain the genetic susceptibility of CLL and the onset of CLL in advanced age. To examine this hypothesis, we studied *Q192R* & *L55M* polymorphisms of *PON1*, a gene implicated in antioxidant pathways, in a large group of CLL patients, in order to investigate the possible contribution of these polymorphisms to CLL development and the formation of CLL chromosomal abnormalities.

Concerning *PON1 Q192R* polymorphism, the frequencies of homozygotes (RR) and heterozygotes (QR) for the mutant allele were significantly increased in the CLL group compared to healthy controls, indicating that individuals with *PON1 Q192R* polymorphism are probably more susceptible to the development of CLL. This was also confirmed by the increased mutant R allele frequency ($p=0.012$) in CLL patients compared

to the control group. Our results are in line with the only study in which *PON1* polymorphisms were investigated in 60 CLL patients (25). In that study, the frequency of both RR genotype and the R allele of the *Q192R* polymorphism was also found statistically significantly increased compared to the control group (25). Additionally, positive relations of this polymorphism have also been found in relative malignancies with CLL such as lymphohematopoietic cancers and lymphomas (32, 33). In another study it was observed that the RR mutant genotype of the *Q192R* polymorphism is associated with follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) and with increased susceptibility to lymphoma in general (34). This observation is very interesting as both FL and DLBCL belong to the same hematological group as CLL entitled "Chronic Lymphoproliferative Diseases" according to WHO 2016 Classification. The above, combined with the fact that approximately 10% of individuals with CLL report a family history of CLL or a related lymphoproliferative disorder (35), may suggest a common pathogenetic mechanism including common genetic predisposition factors for Chronic Lymphoproliferative Diseases. Moreover, *PON1 Q192R* polymorphism has been associated with an increased risk of lung cancer and has been suggested as a clinically useful genetic marker for small cell and squamous cell carcinoma (26).

In the current study, *Q192R* genotypes and allele frequencies were associated for the first time with the cytogenetic findings of CLL patients. This association revealed a significantly increased frequency of this polymorphism in patients with abnormal karyotypes and in patients with *abn(14q32)*, *del(6q)*, and *del(11q)* indicating the possible role of *PON1 Q192R* polymorphism in the formation of specific CLL chromosome aberrations, which are implicated in CLL pathogenesis. This finding requires further study since it has been previously shown that B-cells from CLL patients with certain cytogenetic structural aberrations, such as *del(17p)* and *del(11q)*, were more susceptible to injury caused by reactive oxygen species (ROS) (36). Based on the above and in combination with the facts that accumulation of ROS results in oxidative stress, which contributes to genomic instability, and that a portion of CLL patients acquire new aberrations during the course of the disease, we suggest that increased levels of ROS due to *PON1* polymorphisms could promote oxidation-mediated mutagenesis contributing to accelerated malignant progression.

Concerning *PON1 L55M* polymorphism, genotypic and allelic frequency distributions between CLL patients and healthy controls did not significantly differ, indicating that *PON1 L55M* polymorphism does not confer a genetic predisposition to CLL. Our results are differentiated from those of Tomatir *et al.* reporting an increased frequency of MM genotypes in CLL patients compared to controls, probably due to the small number of CLL patients (60 patients) in that study (25). Moreover, *M55L* polymorphism

was never positively associated with other hematological malignancies although it had been previously examined as a predisposing factor in other types of malignancies, such as breast cancer, lung cancer and colorectal cancer (27, 34, 37, 38). More specifically no association was found between *PON1* L55M polymorphism and colorectal cancer risk or lung cancer risk (26-27). Stratification of CLL patients according to their cytogenetic findings and *PON1* M55L genotypes and alleles failed to reveal a positive association of this polymorphism with CLL chromosomal abnormalities.

Today, it is widely accepted that minor genetic changes, such as genetic variation in the human genome, which affect cell detoxification or DNA repair, may contribute to CLL genetic susceptibility (36, 39-42). It is also known that CLL risk increases and the antioxidant capacity of the human body decreases with age. Moreover, it has been reported that CLL and NHL patients ≥ 65 years encounter more toxicities than younger patients after chemotherapy due to reduced antioxidant capacity (43). Based on the above and our findings, we suggest that the Q192R polymorphism of the fundamental antioxidant *PON1* gene, which results in the reduction of the antioxidant *PON1* activity, along with the accumulation of genotoxic compounds due to the onset of CLL in advanced age, may play a role in CLL development and in the formation of CLL chromosomal abnormalities. Recent studies have shown that treatment of CLL cells using antioxidants in combination with existing therapeutic strategies could neutralize endogenous ROS, protect the immune system and improve survival (36, 44). Therefore, in order to improve survival of CLL patients using antioxidants, especially of those patients with reduced detoxification capacity, such as patients with *PON1* polymorphisms, the screening of their genetic background for such genes should be pursued.

In conclusion, our study showed that *PON1* Q192R polymorphism may be implicated in CLL predisposition and the formation of specific CLL chromosomal aberrations.

Conflicts of Interest

The Authors have no conflict of interest to disclose regarding this study.

Authors' Contributions

A. Ioannidou collected patients' data, performed the molecular experiments, wrote the manuscript and contributed to the revision of the manuscript; S. Zachaki performed the statistical analysis and contributed to interpretation of the results, writing and revision of the manuscript; A. Daraki contributed to the design of the molecular part of the study; I. Margariti, D. Pantelia and C. Sambani contributed to the karyotypic analysis; P. Diamantopoulou contributed to the karyotypic and FISH analysis; P. Roussou provided samples and clinical data of CLL patients; K. N. Manola designed the research, contributed to karyotypic and FISH analysis,

interpretation of results, writing and revision of the manuscript. All Authors read and approved the final manuscript.

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