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Running title: Frequency of HLA class I alleles in Iranian CLL patients

Abstract

Chronic lymphocytic leukemia (CLL) is a malignant disorder of B cell origin, with low incidence in Asian populations. In this study we investigated the HLA-class I A and B allele frequencies in 87 Iranian CLL patients and 64 healthy controls using sequence specific primer-polymerase chain reaction (SSP-PCR) technique. Our results showed increased frequencies of HLA-A11:01 ($p=0.02$) and HLA-B35:01 ($p=0.002$) alleles and HLA-A11:01/B35:01 haplotype ($p=0.036$) and decreased frequencies of HLA-A01:01 ($p=0.02$), HLA-A26:01 ($p=0.03$), HLA-B65:01 ($p=0.03$) and HLA-B53:01 ($p<0.00001$) alleles in CLL patients compared to the control group. Classification of the patients into non-progressive and progressive groups did not reveal significant differences for the frequency of any of the HLA-A and B alleles or haplotypes between these two subtypes. Comparison between patients with immunoglobulin heavy chain variable region genes (IGHV) mutated (n=56) and unmutated (n=31) subtypes showed a significant increase in HLA-A32:01 ($p=0.05$) and HLA-A33:01 ($p=0.05$) alleles in IGHV unmutated patients compared to IGHV mutated patients. Similarly, a higher frequency of HLA-B52:01 ($p=0.037$) alleles was observed in CD38+ compared with CD38- patients. Our results obtained from an Iranian population indicate that CLL is associated with distinct HLA class I alleles and haplotypes some of which are linked to disease prognostic factors.

Introduction

Chronic lymphocytic leukemia (CLL), a hematologic malignancy of $CD5^+$ $CD19^+CD23^+$ B-cells [1], is considered as the most abundant leukemia in the West [2, 3]. Compared to the Western populations, its frequency is low in Asia and Iran [4-6]. Clinically, the disease could either be progressive or non-progressive with overall survival time ranging from less than 5 years to more than 30 years. Despite recent identification of several prognostic factors [7, 8] the etiology of CLL remains obscure. Recent investigations on the immunoglobulin heavy chain variable region (IGHV) genes stereotypes of the leukemic B cells suggest involvement of antigen selection in initiation or progression of CLL [9, 10]. Sequencing of the expressed IGHV genes has shown that patients with CLL can be classified into IGHV mutated or unmutated subtypes [11]. Patients having leukemic cells with unmutated IGHV genes usually display a more aggressive disease compared to those with mutated IGHV genes [12, 13].

HLA genes are the most polymorphic genes which are involved in antigen presentation to T lymphocytes. Expression of certain HLA class I or II alleles has been shown to be associated with a variety of infections and autoimmune or malignant disorders [14-16].

Taking into consideration the genetic diversity and variability of HLA alleles in different ethnic populations, different HLA class I and II alleles might be associated to a given disease in different populations.

Association of certain HLA class I [17] or HLA class II [14, 18] alleles with the clinical course of the disease has also been reported in different malignancies.

Apart of a few studies [4, 16, 19-26] in which the frequency of HLA alleles has been determined in CLL patients from some ethnic populations, association of HLA alleles and CLL prognosis has

not been well studied, especially in the Asian CLL patients. In a prior study we reported the frequency of some HLA class I A, B and C antigens in a limited number of CLL patients using the serological microlymphocytotoxicity technique [4]. Later, we reported the HLA class II allele frequencies in a large number of Iranian CLL patients and their correlation to disease progression and IGHV mutation [14]. In the current study, we investigated the frequency of HLA-class I A and B alleles and haplotypes by SSP-PCR technique in the same set of patients. Contrary to our previous serologic HLA class I study, the patients were classified into different molecular and clinical subtypes (progressive and non-progressive, IGHV mutated and unmutated, as well as CD38+ and CD38- subtypes) which allowed analysis of the association of HLA alleles and haplotypes with disease progression.

Materials and Methods

Patients and controls

In the present study, from 87 Iranian CLL patients, 20 ml of heparinized peripheral blood was collected. All patients attended the Hematology and Oncology Clinics of Vail-Asr and Firozgar Hospitals, affiliated to Tehran University of Medical Sciences. Consent letter was taken from all patients and the study was approved by the Ethical Committee of Tehran University of Medical Sciences. CLL diagnosis and classification of our patients have been described previously [14]. Patients were considered to have progressive disease if the following criteria were met: progression during the preceding 3 months in disease-related anemia (hemoglobin <100 g/l), thrombocytopenia (<100 x 109/l) and/or an increase in spleen/liver/lymph node size and/or more than a 2-fold increase in the blood lymphocyte count [14, 27, 28]. HLA typing results were compared with those obtained from 64 Iranian healthy blood donors. Since the age of the subjects does not seem to influence HLA frequency profile, the control group was not age matched with CLL patients and was selected from adult volunteers (23-45 years old; mean age: 34 years).

Typing of HLA-A and HLA-B alleles

HLA class I alleles of patients and controls were defined using low-resolution kits supplied by Qiagen Vertriebs GmbH (Vienna, Austria), based on the manufacturer's instructions.

To define the HLA-A and -B alleles in patients and controls, genomic DNA of each sample was amplified by 24 and 48 cycles of PCR reactions for HLA-A and HLA-B specificities, respectively. Allele frequencies in CLL patients and controls were calculated by direct counting. Two-locus haplotype (HLA-A/B) frequencies in patients and healthy controls were estimated by maximum-likelihood method according to the expectation-maximization algorithm using Arlequin 2.0 [29].

Analysis of *IGHV* genes mutations

Total cellular RNA was isolated from PBMCs of CLL patients using RNA-Bee (BioSite, Taby, Sweden) based on the guanidine thiocyanate phenol chloroform extraction method. First strand cDNA was synthesized from 1–3 ug of total RNA as described previously [14]. To determine the IGHV family gene expression by polymerase chain reaction (PCR), serial dilutions of DNA and cDNA (1:10 to 1:2000) were prepared and PCR amplification was performed using IGHV family-specific degenerative primers. PCR reactions were performed as described [14]. Finally clonal PCR products were purified by excision using the QIAquick gel extraction kit (Qiagen, Hilden, Germany) and were cloned into pGEM-T easy vector (Promega, Southampton, United Kingdom). Sequencing was performed from both directions using the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA), and T7 and SP6 primers. For each sample IGHV gene was identified by matching to the closest known human germline gene using the ImMuno-Gene Tics (IMGT) Database (http://imgt.cines.fr) and the IgBLast search (http://www.ncbi.nlm.nih.gov/igblast/). Classification of patients into mutated (n = 56) and unmutated (n = 31) subtypes was based on more than 98% homology in nucleotide sequence of IGHV genes of the leukemic cells [30].

Statistical analysis

The association of CLL with HLA-I alleles was analyzed by comparing HLA-A and -B alleles frequencies in CLL patients with 64 healthy Iranian controls. Maximum likelihood method was used to evaluate the haplotype frequencies for the two-loci. Chi-Square test for 2×2 tables after Yates correction was used to define the differences between allele frequencies in patients and controls and patients subgroups, using Epi-Info and SPSS statistical packages (SPSS Inc.,

Chicago, IL). The odds ratios (OR) with 95% confidence intervals (CI) were calculated and p-values lower than 0.05 were considered to be significant. The Mann–Whitney U test was also used to compare the time to first treatment (TTFT), progression free survival (PFS) and overall survival (OS) between different CLL subtypes.

Results

HLA-A and B frequencies in CLL patients and controls

In the current study, 87 CLL patients and 64 healthy donors were included. The frequencies of HLA-A and HLA-B alleles in all patients and controls are presented in Tables 1 and 2. Our results showed that HLA-A02 and HLA-A24 have the highest frequency among HLA-A alleles in CLL patients (25.3% and 16.7%) and controls (15.6% and 16.4%), respectively. HLA-A03 and HLA-A11 are the next alleles with higher frequencies in CLL patients. Comparing the frequency of HLA-A alleles between patients and controls shows substantially higher frequencies of HLA-A11 (OR=3.38, p=0.02) and HLA-A02 (OR=1.83, p=0.059) and lower frequencies of HLA-A01 (OR=0.34, p=0.02) and HLA-A26 (OR=0.32, 0.03) in CLL patients (Table 1). The most abundant HLA-B allele in patients was HLA-B35 .This alleles was expressed in CLL patients at a significantly higher frequency compared to controls (19% vs. 6.3%, OR=3.51, p=0.002). On the other hand HLA-B53 allele was among the least expressed HLA-B alleles in CLL patients, but the most frequent allele expressed in the control subjects (OR=0.04, p=0.00001). HLA-B65:01 was also observed at significantly lower frequency in patients compared to controls (OR=0.17, p=0.03) (Table 2).

Frequencies of HLA-I alleles in CLL subtypes

The association of HLA-A and HLA-B alleles to disease progression was analyzed in CLL patients. The frequencies of HLA-A02:01, HLA-A24:01 and HLA-A03:0 alleles were lower in progressive CLL patients, but the differences were not statistically significant. Comparison of HLA-A allele frequencies between IGHV mutated and unmutated CLL patients showed a higher frequency of HLA-A32:01 (6.5% vs. 0.9%, OR=0.13, p=0.05) and HLA-A33:01 (6.5% vs. 0.9%, OR=0.13, p=0.05) in IGHV unmutated compared to IGHV mutated patients. Comparison of

$CD38^+$ and $CD38^-$ groups of patients demonstrated a positive association of HLA-A30:01 and HLA-A68:01 alleles with CD38 expression (OR=3.5, p=0.09 and OR=7.9, p=0.06 respectively), but the differences were not statistically significant.

The frequencies of HLA-B alleles were analysed in CLL subtypes. Significant differences were observed neither between non-progressive and progressive nor IGHV mutated and unmutated CLL subgroups. A significantly higher frequency of HLA-B52:01 was observed in $CD38^+$ compared to $CD38^-$ CLL patients (10.4% vs. 2.4%, OR=4.77, p=0.037). The most frequent homozygote alleles of HLA-A and HLA-B were HLA-A24:01 (8.5%) and HLA-B35:01 (6.9%) in CLL patients (Table 3). None of the controls expressed HLA-B35:01 homozygote allele, but the difference between CLL patients and healthy controls was not statistically significant.

HLA-A and -B haplotypes frequency

The frequencies of two loci haplotypes in CLL patients and healthy controls are shown in Table 4. HLA-A02:01/B35:01, HLA-A24:01/B35:01, HLA-A03:01/B35:01 and HLA-A11:01/B35:01 were the most frequent haplotypes in patients, however, only the frequency of the latter haplotype was significantly higher in the patients compared to the control group (p=0.036). None of the haplotypes was found to be associated to a particular subtype of CLL patients.

Association between TTFT, PFS and OS with HLA I alleles

Comparison of TTFT, PFS and OS in our CLL patients showed no significant correlations between these parameters and frequency of certain HLA-A or -B alleles.

Discussion

Association between HLA antigens and disease initiation or progression has been investigated in a limited number of hematological malignancies, including CLL [14]. The frequency of HLA class I alleles in CLL has been reported in a few ethnic populations (Table 5). Patients with CLL are classified into IGHV mutated and unmutated subtypes based on the mutational status of the IGHV genes of the leukemic B-cells [11]. These two different subtypes of patients have different molecular and clinical features, in terms of CD38 and ZAP-70 expression and disease progression [31, 32]. CLL patients with mutated *IGHV* genes tend to express lower levels of CD38 and ZAP-70 molecules with a milder disease, as compared to patients with unmutated *IGHV* genes [12, 13, 30].

In the current study, we demonstrated the HLA class I A and B alleles frequencies in a group of Iranian CLL patients and healthy controls trying to identify the disease predisposing or protective alleles and haplotypes in our patients. Of the HLA-A alleles, HLA-A11:01 was represented at a higher frequency in our patients compared with controls (OR=3.38, p=0.02), while the frequencies of HLA-A01:01 and HLA-A26:01 alleles were significantly higher in the control group (OR=0.34 and 0.32, p=0.02 and 0.03, respectively). Our results are not compatible with those of Linet et al who reported no difference in the frequencies of HLA-A01 and HLA-A26 alleles between their CLL patients and healthy controls [21], which may partly be due to ethnic differences.

Among the HLA-B alleles we observed a significantly higher frequency of HLA-B35:01 allele in our CLL patients compared to controls (OR=3.51, p=0.002), showing a strong association of this allele with CLL disease. A similar finding was also reported by others [20], though controversial

results have also been published [16, 21, 22, 25, 33]. An increased frequency of the HLA-B35 allele has also been reported in some other malignancies and autoimmune diseases from different ethnic populations, including Hodgkin's lymphoma [34], autoimmune thyroiditis [35], autoimmune hepatitis [36] autoimmune arthritis [37], Moyamoya [38], and pemphigus vulgaris [39], suggesting a critical correlation of this allele with hematologic malignancies and other pathologic conditions.

In contrast to higher frequency of HLA-B35:01 in CLL patients, a significantly lower frequency of HLA-B65:01 (OR=0.17, p=0.03) and particularly HLA-B53:01 (OR=0.04, p=0.00001) alleles was observed in our patients compared to the healthy controls, suggesting a protective role for these alleles. To our knowledge negative association of these two alleles with CLL has not been reported in other ethnic populations, suggesting ethnical influence of HLA association to CLL disease.

The differences observed in allele frequencies between patients and controls might be interpreted to reflect differences between different subgroups of the Iranian population. Ethnically, the Iranian population is derived from the Caucasian ancestral, though it is composed of different ethnic subpopulations, including Turk, Kurd, Arab, Fars, Balooch, Lor and Turkman people [40]. These populations are living in different provinces of Iran. However, Tehran, the capital of Iran, is populated with all these subgroups. Thus, since the samples were collected from patients and personnel of two university hospitals located in Tehran, we assume that it is unlikely that the differences observed in allele frequencies between our patients and controls are associated to population subgroups.

Comparison of the frequency of HLA-A alleles in different subtypes of our CLL patients showed a significantly higher frequency of HLA-A32:01 and HLA-A33:01 alleles in IGHV unmutated compared to mutated patients.

A higher frequency of HLA-A33 allele has previously been reported in patients with other disorders [41, 42]. Among the HLA-B alleles, HLA-B52:01 allele was expressed at a higher frequency in CLL patients expressing CD38 molecule, compared to $CD38^-$ patients (OR=4.77, p=0.037). Association between expression of CD38 and/or lack of IGHV mutation with disease progression has been reported in various studies [7, 43, 44]. Accordingly, HLA-A32:01, A33:01 and B52:01 alleles seem to be positively associated to disease progression in our CLL patients. As we reported previously [14], non-progressive and IGHV mutated CLL patients had a higher PFS (44 and 37 months, respectively) compared to progressive and unmutated (13 and 9 month, respectively) CLL patients (p=0.006 and 0.001, respectively). Also, IGHV unmutated CLL patients had significantly lower overall survival compared to mutated patients (98% vs. 84%, p=0.04). Our findings of lack of significant correlations between TTFT, PFS and OS with frequency of expression of none of the HLA-A or –B alleles in our CLL patients suggest that different prognostic parameters may give different levels of associations with HLA alleles frequency.

The negative association of certain HLA-I antigens such as HLA-B53:01 with CLL implies expansion of a repertoire of tumor-specific CTL clones which enables effective immunomonitoring and control of tumor cells in healthy subjects. The association of some HLA alleles or haplotypes with CLL disease and its progression may also confer a protective role in CLL in which the leukemic cells and/or antigen presenting cells can efficiently present tumor related antigens to autologous T cells, leading to their stimulation. Differential frequency of some HLA class I alleles in different subtypes of our CLL patients is in agreement with this proposition.

Altogether, our findings together with the signaling effects of HLA-I molecules and effects on tumor cells behavior imply that certain HLA antigens may influence the immune response to leukemic CLL cells, probably through different mechanisms such as presentation of a tumor-associated antigen by leukemic B cells or by professional antigen-presenting cells to autologous T cells and shaping the repertoire of T cells as explained. Alternatively, such alleles may be regarded as markers for other pathogenic genes remaining in linkage disequilibrium with these loci.

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Table 1: HLA-A allele frequencies among Iranian CLL patients and healthy controls.

Alleles	CLL (n=87) (174 alleles)	Healthy controls (n=64) (128 alleles)	Odds Ratio (95% CI)	P value
A02:01	44 (25.3) [a]	20 (15.6)	1.83 (0.98-3.43)	**0.059**
A24:01	29 (16.7)	21 (16.4)	1.02 (0.53-1.97)	0.92
A03:01	26 (14.9)	18 (14.1)	1.07 (0.54-2.16)	0.96
A11:01	21 (12.1)	5 (3.9)	3.38 (1.16-10.55)	**0.02**
A01:01	8 (4.6)	16 (12.5)	0.34 (0.13-0.87)	**0.02**
A32:01	8 (4.6)	7 (5.5)	0.83 (0.27-2.63)	0.93
A29:01	7 (4)	3 (2.3)	1.75 (0.45-6.51)	0.63
A30:01	7 (4)	5 (3.9)	1.03 (0.33-3.17)	0.8
A26:01	6 (3. 4)	13 (10.2)	0.32 (0.1-0.92)	**0.03**
A33:01	5 (2.9)	8 (6.3)	0.44 (0.12-1.54)	0.25
A68:01	4 (2.3)	6 (4.7)	0.48 (0.11-1.96)	0.41
A23:01	2 (1.1)	5 (3.9)	0.29 (0.04-1.69)	0.23
A31:01	2 (1.1)	0	1.12	0.61
A66:01	2 (1.1)	0	1.12	0.61
A34:01	1 (0.6)	0	NI	0.87
A69:01	1 (1.1)	0	NI	0.87
A74:01	1 (1.1)	0	NI	0.87
A64:01	0	1 (0.8)	0	0.87

[a] The results represent the number (%) of the specified alleles expressed in the study groups, NI= not identified, CI = confidence interval (lower limit–upper limit).

Table 2: HLA-B allele frequencies among Iranian CLL patients and healthy controls.

Alleles	CLL (n=87) (174 alleles)	Healthy controls (n=64) (128 alleles)	Odds Ratio (95% CI)	P value
B35:01	33 (19) [a]	8 (6.3)	3.51 (1.48-8.6)	**0.002**
B51:01	17 (9.8)	14 (10.9)	0.88 (0.39-1.98)	0.88
B07:01	10 (5.7)	10 (7.8)	0.72 (0.27-1.94)	0.63
B13:01	9 (5.2)	7 (5.5)	0.94 (0.31-2.9)	0.88
B52:01	8 (4.6)	2 (1.6)	3.04 (0.58-21.07)	0.19
B18:01	7 (4)	1 (0.8)	5.15 (0.64-41.34)	0.14
B38:01	7 (4)	10 (7.8)	0.49 (0.16-1.46)	0.23
B08:01	6 (3.4)	7 (5.5)	0.62 (0.18-2.11)	0.57
B41:01	5 (2.9)	7 (5.5)	0.51 (0.14-1.58)	0.4
B62:01	5 (2.9)	2 (1.6)	1.86 (0.36-9.33)	0.71
B44:01	4 (2.3)	4 (3.1)	0.73 (0.15-3.54)	0.94
B50:01	4 (2.3)	2 (1.6)	1.48 (0.27-7.91)	0.97
B55:01	4 (2.3)	9 (7)	0.31 (0.08-1.14)	0.08
B27:01	3 (1.7)	1 (0.8)	2.23 (0.23-20.97)	0.84
B40:01	3 (1.7)	3 (2.3)	0.73 (0.12-4.62)	0.97
B49:01	2 (1.1)	4 (3.1)	0.36 (0.05-2.33)	0.42
B58:01	2 (1.1)	1 (0.8)	1.48 (0.13-16.05)	0.79
B63:01	2 (1.1)	3 (2.3)	0.48 (0.08-2.89)	0.72
B65:01	2 (1.1)	8 (6.3)	0.17 (0.03-0.91)	**0.03**
B15:01	1 (0.6)	0	NI	0.87
B37:01	1 (0.6)	1 (0.8)	0.73 (0.05-11.65)	0.61
B45:01	1 (0.6)	2 (1.6)	0.36 (0.03-4.01)	0.79
B53:01	1 (0.6)	17 (13.3)	0.04 (0.01-0.32)	**0.00001**
B57:01	1 (0.6)	3 (2.3)	0.24 (0.03-2.33)	0.2
B56:01	0	1 (0.8)	0	0.87
B72:01	0	1 (0.8)	0	0.87

[a] The results represent the number (%) of the specified alleles expressed in the study groups, NI= not identified, CI = confidence interval (lower limit–upper limit).

Table 3: HLA-A, B homozygote alleles frequency in CLL and controls.

Alleles	Homozygote alleles		Odds Ratio (95% CI)	P value
	CLL (n=87)	Healthy controls (n=64)		
A24:01	5 (8.5) [a]	2 (3.1)	**1.89** (0.37-9.18)	0.71
A02:01	3 (3.5)	1 (1.6)	**2.22** (0.23-20.5)	0.85
B35:01	6 (6.9)	0	NI	0.08
B4005:01	2 (2.3)	0	NI	0.61

[a] The results represent the number (%) of the specified alleles expressed in the study groups, NI= not identified, CI = confidence interval (lower limit–upper limit).

Table 4: HLA-A/B haplotype frequency in CLL patients and controls.

Haplotypes	HLA-A/B haplotype frequency in CLL patients	HLA-A/B haplotype frequency in controls	Odds Ratio (95% CI)	P value
A02:01/B35:01	10 (5.7) [a]	3 (2.3)	2.54 (0.69-8.73)	0.25
A24:01/B35:01	10 (5.7)	2 (1.6)	3.84 (0.82-16.5)	0.12
A03:01/B35:01	10 (5.7)	2 (1.6)	3.84 (0.82-16.5)	0.12
A11:01/B35:01	8 (4.6)	0	NI	**0.036**
A02:01/B51:01	5 (2.9)	5 (3.9)	0.73 (0.22-2.49)	0.86
A32:01/B35:01	5 (2.9)	1 (0.8)	3.76 (0.43-31.1)	0.38
A02:01/B07:01	5 (2.9)	0	NI	0.14
A03:01/B51:01	4 (2.3)	5 (3.9)	0.58 (0.16-2.15)	0.64
A26:01/B51:01	4 (2.3)	2 (1.6)	1.48 (0.27-7.91)	0.97
A03:01/B18:01	4 (2.3)	1 (0.8)	2.99 (0.33-26.02)	0.97
A02:01/B41:01	3 (1.7)	3 (2.3)	0.73 (0.15-3.59)	0.97
A02:01/B38:01	3 (1.7)	3 (2.3)	0.73 (0.15-3.59)	0.97
A02:01/B13:01	3 (1.7)	3 (2.3)	1.11 (0.19-6.51)	0.73
A02:01/B18:01	3 (1.7)	1 (0.8)	2.23 (0.23-20.97)	0.84
A26:01/B07:01	3 (1.7)	1 (0.8)	2.23 (0.23-20.97)	0.84
A24:01/B62:01	3 (1.7)	0	NI	0.36
A02:01/B52:01	3 (1.7)	0	NI	0.36
A24:01/B52:01	3 (1.7)	0	NI	0.36
A24:01/B53:01	2 (1.15)	4 (3.12)	0.36 (0.07-1.98)	0.42
A24:01/B55:01	2 (1.15)	4 (3.12)	0.36 (0.07-1.98)	0.42

[a] The results represent the number (%) of the specified alleles expressed in the study groups, NI= not identified, CI = confidence interval (lower limit–upper limit). Data for the most frequent haplotypes are presented in the table.

Table 5: Comparison of HLA class I alleles frequencies in B-CLL patients from different ethnic populations

Country	No. of patients (controls)	HLA-I alleles																									Ref.
		A1:01	A2:01	A3:01	A9:01	A10:01	A11:01	A29:01	A28:01	A30:01	A68:01	B5:01	B7:01	B8:01	B13:01	B15:01	B18:01	B27:01	B35:01	B40:01	B41:01	B44:01	B51:01	B53:01	B55:01	B58:01	
Germany	101 (157) *	24.8 (31.9)	59.4 (48.4)	NI	NI	NI	NI	NI	4 (11.5)	NI	NI	NI	NI	17.8 (21.7)	NI	NI	NI	NI	9.9 (18.5)	NI	NI	NI	NI	NI	NI	NI	16
UK	85 (600)	34 (36)	55 (48)	19 (24)	19 (17)	7 (9)	15 (12)	NI	4 (9)	NI	NI	6 (7)	31 (28)	33 (33)	2 (3)	13 (12)	0 (5)	7 (8)	12 (12)	8 (13)	NI	NI	NI	NI	NI	NI	33
USA	88 (3761)	32 (30)	56 (50)	27 (30)	23 (20)	8 (10)	7 (10)	10 (10)	NI	NI	NI	14 (10)	24 (20)	16 (20)	8 (10)	14 (10)	9 (10)	8 (10)	19 (20)	9 (10)	NI	NI	NI	NI	NI	NI	21
UK	57 (57)	53 (30)	54 (51)	16 (23)	18 (16)	4 (9)	9 (18)	NI	7 (7)	NI	NI	11 (16)	30 (30)	44 (19)	5 (5)	12 (5)	7 (7)	4 (7)	5 (11)	11 (9)	0 (2)	NI	NI	2 (2)	NI	NI	25
UK	**83 (385)**	**30.1 (36.7)**	**62.7 (45.7)**	**NI**	**NI**	**10.8 (7.8)**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**21.7 (26.5)**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**26**
Germany	79 (329)	20.3 (30.4)	60.8 (45.9)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	16.5 (22.2)	NI	NI	11.4 (10.3)	NI	10.1 (15.5)	NI	NI	NI	NI	NI	NI	NI	22
IRAN	87 (64)	4.6 (12.5)	25.3 (15.6)	14.9 (14.1)	NI	NI	12.1 (3.9)	4 (2.3)	NI	4 (3.9)	2.3 (4.7)	NI	5.7 (7.8)	NI	5.2 (5.5)	NI	4 (0.8)	1.7 (0.8)	19 (6.3)	1.7 (2.3)	2.9 (5.5)	2.3 (3.1)	9.8 (10.9)	0.6 (13.3)	2.3 (7)	1.1 (0.8)	Present study

*Data represent the percentage of alleles in CLL (normal controls), NI= not identified