Immunoglobulin Gene Rearrangements and Mutational Status in Argentinian Patients With Chronic Lymphocytic Leukemia

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Abstract

The mutational status and rearrangements of the immunoglobulin heavy chain variable (*IGHV*) gene was analyzed in 73 Argentinian patients with chronic lymphocytic leukemia. Fluorescence in situ hybridization analysis was also performed. Our cohort displayed an *IGHV* gene usage that resembles that observed in Western countries and showed certain differences compared with published series from other Latin American populations.

Background: Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. The mutational status of the immunoglobulin heavy chain variable (IGHV) region represents one of the best prognostic markers and defines 2 disease subgroups: mutated (M-CLL) and unmutated (UM-CLL), with different clinical course. **Materials and Methods:** *IGHV-D-J* gene rearrangements and mutational status were analyzed in 73 Argentinian patients with CLL, 22 previously treated, by reverse transcriptase–polymerase chain reaction and bidirectional sequencing. The results were compared with those reported in other geographic regions. Fluorescence in situ hybridization analysis was also performed. **Results:** A total of 43 (58.9%) cases were of patients with M-CLL, and 30 (41.1%) were patients with UM-CLL. Deletion of chromosome 13q14 as a single alteration was more frequently observed in the M-CLL group (48%) than in the UM-CLL group (24%). In the M-CLL group, the proportion of cases with deletion of chromosome 13q14 was significantly higher than those with +12 and those with deletions of chromosomes 17p and 11q ($P = .003$). The most frequently used IGHV families were IGHV3 > IGHV1 > IGHV4, which are different from those observed in Asian, Brazilian, and Uruguayan series. The *IGHV3-23* gene (10.8%) was the most commonly used, followed by *IGHV1-69* (9.5%), *IGHV4-59* and *IGHV2-5* (6.8% each), and *IGHV3-21* and *IGHV3-30* (5.4% each). *IGHV4-34* showed the lowest frequency (2.7%) in our cohort compared with published data, whereas *IGHV4-59, IGHV3-72,* and *IGHV2-5* were overexpressed in our series. Stereotyped HCDR3 (heavy chain complementary determining region 3) was found in 9.5% of patients. **Conclusions:** Our results showed that Argentinian patients with CLL display an *IGHV* gene usage that resembles that observed in Western countries and exhibited interesting similarities and differences with respect to published series from other Latin American populations, which reflect variations in the genetic background.

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world, accounting for nearly 30% of all leukemias,[1,2] whereas the incidence is low in Asian countries.[3] The clinical course is highly variable, with time to progression, ranging from months to decades.[4] Although clinical staging systems have been very useful in guiding disease management and treatment decisions, additional markers are needed to stratify patients who are at increased risk of disease progression. The mutational status of the immunoglobulin heavy chain variable (*IGHV*) genes,

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phenotypic expression of zeta-associated protein 70, and cluster of differentiation 38, and genomic abnormalities have demonstrated to have independent prognostic utility.[5-9] Particularly, fluorescence in situ hybridization (FISH) analysis allowed the identification of distinct cytogenetic risk groups, in which patients with deletion of chromosome 13q14 as a single alteration have a better outcome, whereas patients with deletions of chromosome 11q22 or chromosome 17p13 show the shortest median survival, and cases with trisomy 12 have an intermediate prognosis.[9]

The mutational status of the *IGHV* genes[5,6] represents one of the best prognostic markers in CLL and defines 2 disease subgroups: one expressing mutated (M) *IGHV* segments, with a more favorable clinical course, and the other expressing unmutated (UM) *IGHV* segments, associated with a poor outcome. CLL displays a remarkably biased *IGHV* gene repertoire, being those that belong to the IGHV1, IGHV3, and IGHV4 families, the most frequently used. Also, somatic mutations are not uniformly distributed among IGHV families that show overrepresentation of IGHV3 and IGHV4 members in the M and overexpression of the IGHV1 family in the UM state.[10,11] In addition, there is compelling evidence that indicates differential expression of some *IGHV* genes in different geographic regions. The most frequently used *IGHV* genes in Western countries are *IGHV1-69, IGHV3-7, IGHV4-34,* and *IGHV3-23,*[12-15] however, a very low frequency of *IGHV1-69* usage is observed in Asian cohorts.[16,17] Moreover, an overrepresentation of the *IGHV3-21* gene has been reported in northern European countries compared with the Mediterranean region.[18,19] Also, a proportion of patients with CLL exhibit closely homologous (stereotyped) heavy chain complementary determining region 3 (HCDR3) sequences, and some of them also carry virtually identical amino acid (aa) sequences,[13,14] which suggests a role for specific antigens in the development of CLL. In reference to Latin America, there is scarce information about the incidence of this pathology,[20,21] and, simultaneously, there are only 3 studies about *IGHV* genes usage in patients with CLL from this region.[22-24]

In this study, we investigated, for the first time to our knowledge, *IGH* rearrangements in Argentinian patients with CLL. The aim of this study was to analyze the *IGHV-D-J* gene usage and the mutational status in our CLL population. Results were compared with those obtained in other geographic regions of the world. The relationship between *IGHV* mutation and genomic abnormalities was also evaluated.

Material and Methods

Patients

Our cohort included 73 unselected patients (49 men; median age, 64.9 years; age range, 36-87 years) with a diagnosis of CLL according to the International Workshop on Chronic Lymphocytic Leukemia Criteria,[25] who were consecutively referred to our institution. Stage was assessed according to the classification of Rai et al.[26] Clinical stages were available in 68 patients, with the following distribution: 0, 24 (35.3%) patients; I, 16 (23.5%); II, 16 (23.5%); III, 4 (5.9%), and IV, 8 (11.8%). Twenty-two (32.3%) patients had received previous treatment. All individuals provided their informed consent. The study was approved by the local ethics committee. There is no national registry of CLL in Argentina, thus we do not have firm data about the incidence and prevalence of this disease in our country.

RNA Extraction and IGHV *Mutational Status*

Total RNA was extracted with Trizol reagent (Invitrogen, Buenos Aires, Argentina) from mononuclear cells isolated on a Ficoll-Paque Plus (GE Healthcare Bio-Sciences, Uppsala, Sweden) density gradient from peripheral blood samples of patients. The complementary DNA synthesis was carried out by using Moloney murine leukemia virus reverse transcriptase and random primer (Promega, Madison, WI). The *IGHV* gene sequences were determined as previously described.[27] Briefly, amplification of *IGHV* regions by polymerase chain reaction was performed on complementary DNA by using VH framework region 1 consensus family specific primers (VH1-VH6) and JH primers. When amplifications of these primers were unsuccessful, an alternative set of primers that anneal to sequences in the leader region (LH1-LH6) and 1 antisense Cμ-primer[28] were used. Thermal cycling conditions were 3 minutes at 93°C, followed by 33 cycles at 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, elongation at 72°C for 7 minutes, and a final step at 4°C for 10 minutes. Polymerase chain reaction products were purified in 2% agarose gels, sequenced bidirectionally, and analyzed on an automated DNA sequence analyzer (377 ABI Prism, PE biosystem, Foster City, CA). Sequence data were analyzed by using IgBLAST (immunoglobulin BLAST) (http://www.ncbi.nlm.nih.gov/igblast) and the ImMunoGeneTics database (IMGT) (http://imgt.cines.fr).[29] *IGHV* sequences with <98% homology with respect to the germline counterpart were considered as M, whereas those with homology of 98% or higher were classified as UM.[5,6] UM sequences were further subdivided according to Murray et al[30] criteria into truly UM (100% identity to the germline), minimally M (99%-99.9% identity), and borderline M (98%-98.9% identity). M sequences were categorized into 2 groups: cases with <96% and cases with 96%-97.9% identity.[19]

For identification of HCDR3 length, IMGT/JunctionAnalysis software (Montpellier, France) was used. For HCDR3-driven clustering, HCDR3 aa sequences between codons 107 and 117 (IMGT numbering) were aligned to published stereotypic clusters[13,14,30,31] by using the multiple sequence alignment software ClustalW.[32] In all patients, *IGHV-D-J* gene segment usage was analyzed, and the mean value of all the pairwise alignment scores (mean alignment score [MAS]) was calculated.[32] MAS values ≥60% were used to include an individual sequence into a cluster.

FISH Analysis

FISH was performed on peripheral blood lymphocytes preparations. The slides were hybridized with Locus Specific Indicator TP53/ATM/13q14/13q34/CEP12 DNA probes (Vysis-Abbott, Downers Grove, IL) according to manufacturer's protocol. For each probe, at least 200 interphase nuclei were analyzed. The cutoff for positive values (mean of normal control + 3 standard deviations), determined from samples of 10 cytogenetically normal donors, were 3.02%, 10.2%, 7.7%, and 5.1% for trisomy 12, monosomies of D13S319, Ataxia Telangiectasia Mutated, and TP53, respectively.

Statistical Analysis

Comparison of frequencies between IGHV families and genes in patients with M-CLL and those with UM-CLL were performed by the χ^2 test and the Fisher exact test by using the SPSS package (SPSS Inc, Chicago, IL). Quantitative variables were compared by using the Student *t* test. For all tests, $P < .05$ was considered as statistically significant.

Results

Age, sex, clinical stage, mutation status, *IGHV-D-J* rearrangements, and FISH characteristics of 73 patients with CLL are summarized in Table 1. Forty-three (58.9%) cases were M, whereas the remaining 30 (41.1%) were classified as UM. One patient showed double in-frame rearrangements (case 9). Furthermore, among UM sequences, 25/31 (80.6%) were truly UM 4/31 (12.9%) were minimally M, and 2/31 (6.5%) were borderline M. In addition, 35/43 (81.4%) patients with M state showed sequences with <96% identity to the germline and 8/43 (18.6%) had 96%-97.9% identity. The analysis of clinical stages showed a higher proportion of patients with M-CLL and with Rai 0 17/41 (41.4%) compared with UM-CLL cases 7/27 (25.9%). The distribution of mutational status was similar among previously treated patients (50%), meanwhile 66.7% of untreated patients were M and 33.3% were UM. Only 1 patient (case 27) showed evolution to the Richter transformation, and 3 cases had autoimmune manifestations (cases 6, 18, and 42). FISH studies were performed in 61 cases. Deletion of chromosome 13q as a single alteration was more frequently observed in patients with M-CLL 16/33 (48.5%) compared with the UM-CLL subgroup 6/25 (24%). When the M-CLL group was analyzed, significant differences in the proportion of cases with del(13q14) as a single alteration compared with +12 4/33 (12%) ($P = .003$) and deletion of chromosomes 17p and 11q 4/34 (11.7%) ($P = .003$) were found. FISH results did not show differences in the distribution of genomic rearrangements among treated and untreated patients.

The most commonly expressed IGHV family was IGHV3 39/74 (52.7%), followed by IGHV1 17/34 (23%), IGHV4 12/74 (16.2%), IGHV2 5/74 (6.7%), and IGHV7 1/74 (1.4%), with no expression of IGHV5 and IGHV6 (Figure 1A). IGHV3 and IGHV4 families were predominant among patients with M-CLL (66.7% and 75%, respectively). In contrast, 76.5% of patients expressing IGHV1 displayed UM status, with significant difference compared with IGHV3 ($P = .0039$), IGHV4 ($P = .0095$), and the entire series ($P = .0016$).

Regarding *IGHV* gene usage, 28 segments were represented in our cohort. *IGHV3-23* 8/74 (10.8%) and *IGHV1-69* 7/74 (9.5%) were the most frequently used segments, followed by *IGHV2-5* and *IGHV4-59* 5/74 (6.8% each), and *IGHV3-30* and *IGHV3-21* 4/74 (5.4% each) (Figure 1B). Collectively, these genes were found in 44.7% of the evaluated cases. Among the family members, *IGHV1-69* was UM in 6 (85.7%) of 7 patients, all of them with 100% sequence homology, whereas *IGHV3-72* and *IGHV3-7* were always related to M status. In addition, *IGHV4-59* and *IGHV3-23* genes appeared to be mostly M (80% and 75% of patients, respectively), all of them with <96% sequence homology.

Twenty-two different Immunoglobulin Heavy Chain Diversity *(IGHD)* segments were used in our series. The frequencies of family usage were *IGHD3* 32/74 (43.2%), *IGHD2* 14/74 (18.9%), *IGHD6* 10/74 (13.5%), *IGHD1* 8/74 (10.8%), *IGHD5* 7/74 (9.5%), and *IGHD4* 3/74 (4.1%) (Figure 2A). *IGHD3-3* 16/74 (21.6%) was the most common segment used, followed by *IGHD3-10* 7/74 (9.5%), *IGHD3-22* 6/74 (8.1%), *IGHD1-26,* and *IGHD2-21* 5/74 (6.8% each). As previously reported,[11,33] an overusage of *IGHD3* family in the UM-CLL group (61.3%) was observed, which showed significant differences with respect to the whole series ($P = .0097$) as well as *IGHD3-3* segment (13/31 [42%]) ($P = .0005$) (Figure 2B). Most of patients with *IGHD3-22* (5/6 [83.3%]) were in the M-CLL group.

As for *IGHJ* segments, *IGHJ4* was the most frequently used 33/74 (44.6%), followed by *IGHJ6* 19/74 (25.7%), *IGHJ3* 12/74 (16.2%), *IGHJ5* 7/74 (9.5%), and *IGHJ1* 3/74 (4.0%), with no representation of *IGHJ2* (Figure 3A). This is in contrast to other series in which *IGHJ5* was more commonly used than *IGHJ3*.[10,12] Confirming previous analysis,[10,11,34] *IGHJ4* segment was preferentially expressed among the M-CLL group 24/33 (72.7%), whereas *IGHJ6* was more frequently used in UM-CLL patients 12/19 (63.2%) with significant differences to the whole series ($P = .033$). In addition, the *IGHJ4*02* segment was the most commonly expressed 29/74 (39.1%), followed by *IGHJ6*02* 16/74 (21.6%) and *IGHJ3*02* 11/74 (14.9%) (Figure 3B).

The analysis of HCDR3 sequences showed a median length of 16 aa (range, 6-27) for all samples and a significant shorter size in the M group (14 aa; range, 6-27) compared with UM patients (19 aa; range, 11-25) ($P = .0001$) (Table 1). The size was also longer in IGHV1 (19 aa) than in IGHV2 (15 aa) ($P = .008$), IGHV3 (15 aa) ($P = .004$), and IGHV4 (15 aa) ($P = .035$) expressing samples. Coincidentally, the mean length of the *IGHV1-69* gene (22 aa) associated with the UM state was significantly longer than *IGHV3-7* and *IGHV3-72* (12 aa each) ($P = .004$ both), *IGHV2-5* (15 aa) and *IGHV3-23* (16 aa), all preferentially associated with the M state. Comparative analysis of the HCDR3 aa sequences in our series showed stereotyped HCDR3 in 7 (9.5%) patients (Table 2); 5 of 7 cases expressed UM *IGHV* genes with 100% identity to the germline. Four patients were associated with the IGHV1 family and the remaining 3 belonged to the IGHV3 family: *IGHV3-21* (1 case) and *IGHV1-69, IGHV3-30,* and *IGHV1-3* (2 cases each). At the time of this study, 3 of these patients (cases 16, 18, and 26) had received treatment, with time to first treatment of: 49 months, 6 months, and 1 month, respectively.

Discussion

In this study, *IGHV* gene usage and mutational status have been analyzed for the first time in Argentinian patients with CLL. The frequencies of cases with M and UM *IGHV* genes were similar to those reported for Western populations.[10,11,13] FISH analysis showed an association between del(13q14) as a single alteration and the M-CLL subgroup, which supports the more favorable outcome of both parameters. In this context, our data confirm previous results of the literature that indicate a different distribution of genomic rearrangements in patients with M-CLL and those with UM-CLL.[8,35]

IGHV family distribution in our cohort was comparable with those observed in Western countries (Table 3),[10,13,36] which showed

Table 1 Clinical Features, Mutational Status, *IGHV* Gene Rearrangements, Cytogenetics and FISH Analysis in 73 Patients with Chronic Lymphocytic Leukemia

Case No.	Age (y)/ Sex	Clinical Stage	MS (% Homology)	IGHV Family	IGHV Gene	IGHJ Segment	IGHD Segment	HCDR3 (bp)	FISH (%)			
									del13q14	+12	del17p13	del11q22
1	57/M	II	UM (100)	3	V3-11*01	J3*02	D3-3	18	2.3	0.8	1.7	2.04
2	79/F	II	M (94.3)	3	V3-43*01	J4*02	D4-17	11	1.1	35.4	0.7	2.1
3	72/F	ND	UM (100)	2	V2-5*01	J4*02	D5-5	12	ND	ND	ND	ND
4	54/M	I	UM (100)	4	V4-31*03	J6*02	D3-3	23	2.4	0	1.7	1
5	77/F	I	M (97.4)	1	V1-18*01	J4*03	D3-16	12	0	0	0	1.6
6	70/M	0	M (95.0)	3	V3-72*01	J6*02	D1-26	15	X1: 92/X2: 4.4	0	0.9	2.4
7	80/M	IV	M (95.8)	3	V3-23*01	J1*01	D6-19	11	ND	0	45.3	9
8	74/M	I	M (92.0)	1	V1-2*02	J4*02	D3-3	13	ND	0	0.35	0.7
9	65/F	II	UM (100)	3	V3-33*01	J3*02	D2-2	19	1.3	0	1	0.8
			UM (100)	1	V1-46*01	J4*02	D6-13	17				
10	78/M	0	UM (100)	7	V7-4*01	J5*02	D5-12	15	5.5	1.2	0.9	2.5
11	72/F	0	UM (99.3)	1	V1-59*01	J6*02	D3-3	23	2.3	1.6	4.8	2.3
12	57/F	0	UM (99.0)	3	V3-66*01	J4*01	D2-21	14	47.8	0	0.4	1
13	55/F	0	M (95.3)	4	V4-59*08	J4*02	D3-22	17	X1: 67.4/X2: 14	0	2	0.6
14	55/F	I	UM (100)	1	V1-3*01	J4*02	D6-19	13	2.2	1.9	0.4	0.3
15	80/F	0	M (94.4)	3	V3-30*03	J3*02	D1-20	14	62.8	0	0.4	3.7
16	73/M	I	UM (100)	1	V1-3*03	J6*02	D1-26	17	0.5	57.8	0.95	0
17	40/M	0	M (90.9)	3	V3-21*04	J4*02	D5-18	16	19.3	0.4	3	2.4
18	61/M	II	UM (100)	1	V1-69*01	J6*02	D3-3	24	X1: 79.6/X2: 4.9	0	0	1.3
19	77/M	III	M (86.8)	3	V3-23*01	J4*02	D3-3	13	0.9	0	14	0.9
20	83/F	II	M (91.0)	3	V3-7*03	J5*02	D6-13	10	94.2	0	0.4	0
21	66/F	0	M (93.2)	3	V3-72*01	J4*02	D5-24	11	4.03	0	1.7	0
22	67/M	0	M (85.7)	3	V3-23*01	J4*02	D3-10	13	1.35	0	0	0
23	67/M	I	M (90.6)	4	V4-61*06	J4*02	D1-1	11	57.5	0	0	0
24	65/M	0	UM (100)	3	V3-48*03	J5*02	D3-22	19	0	0	0	24.4
25	39/M	II	M (96.0)	2	V2-5*10	J4*02	D3-10	15	57	0	0	0
26	51/M	IV	UM (100)	3	V3-30*03	J6*02	D3-3	24	88.4	0	93.5	0
27	62/M	II	UM (100)	4	V4-59*02	J5*02	D3-3	24	0.9	0	0	0
28	80/M	0	M (91.0)	3	V3-72*01	J3*02	D3-22	12	2	0	5.0	0
29	53/M	II	UM (100)	1	V1-59*01	J6*02	D3-3	25	19.6	0	1.3	0.5
30	52/M	III	UM (98.0)	4	V4-4*07	J4*02	D3-3	12	X1: 44/X2: 22	56.4	0	0
31	59/M	0	M (93.0)	3	V3-74*01	J3*02	D4-23	15	62	0	1	1.6
32	65/F	0	M (95.0)	4	V4-59*03	J4*01	D6-13	10	0	0	0	3.8
33	71/F	0	UM (99.0)	3	V3-23*01	J5*02	D3-10	24	X1: 67.7/X2: 3.2	0	0	1.8
34	66/M	0	M (91.7)	3	V3-33*01	J4*02	D3-10	13	49	0	0	0
35	47/M	I	M (87.9)	4	V4-59*07	J6*02	D2-15	10	71.3	0	0	1.2
36	54/F	IV	UM (100)	1	V1-58*01	J3*02	D3-16	20	0	0	1.4	0
37	46/M	III	M (96.2)	3	V3-9*01	J4*02	D3-3	12	83	0	0	0
38	69/M	IV	M (93.0)	3	V3-15*01	J4*02	D2-21	12	X1: 16.9/X2: 8.4	0.4	0	0.5
39	76/M	0	M (89.0)	3	V3-11*01	J4*02	D1-26	10	ND	ND	ND	ND

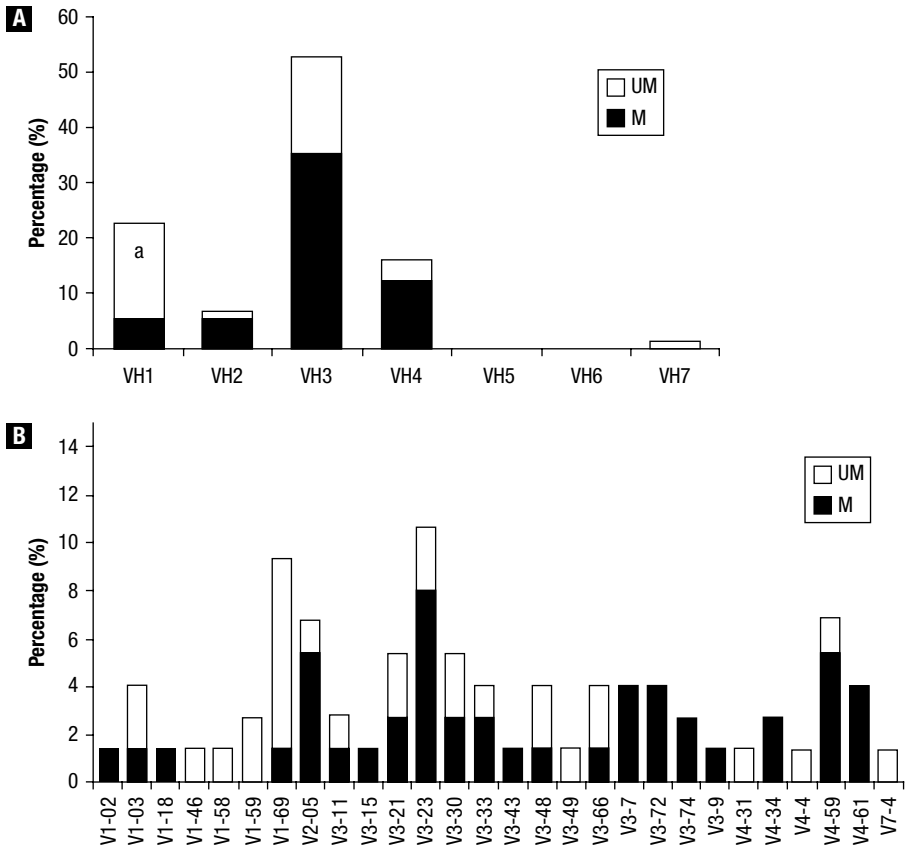
Table 1 (continued)

Case No.	Age (y)/ Sex	Clinical Stage	MS (% Homology)	IGHV Family	IGHV Gene	IGHJ Segment	IGHD Segment	HCDR3 (bp)	FISH (%)			
									del13q14	+12	del17p13	del11q22
40	75/F	I	M (97.3)	3	V3-66*01	J6*03	D3-22	18	5.16	40.4	0	0.9
41	76/M	II	M (88.0)	4	V4-34*01	J6*02	D2-15	24	ND	ND	ND	ND
42	59/M	II	UM (100)	3	V3-23*01	J4*02	D3-10	23	ND	ND	ND	ND
43	51/M	I	UM (100)	3	V3-49*03	J4*02	D3-3	21	0	0	73.9	0
44	60/M	II	UM (100)	1	V1-69*01	J6*02	D3-10	24	15.5	0	58.6	0
45	69/M	0	M (92.0)	3	V3-7*03	J4*02	D2-8	14	90	0	0	0
46	60/M	II	M (93.5)	3	V3-7*01	J3*02	D6-16	14	X1: 48.6/X2: 7.78	ND	1.5	ND
47	53/M	III	M (90.3)	3	V3-23*01	J4*02	D2-15	14	0	35	0	0.8
48	75/M	ND	UM (100)	3	V3-30*03	J6*04	D3-16	18	ND	ND	ND	ND
49	73/F	II	UM (100)	1	V1-69*13	J5*02	D2-2	16	0	49.5	0.9	0.5
50	38/M	II	UM (100)	3	V3-21*02	J6*02	D5-12	22	90	0.4	0.5	0.5
51	60/M	0	M (96.5)	4	V4-61*02	J4*02	D4-23	12	86.7	0	0	0
52	78/M	I	M (88.0)	3	V3-23*01	J4*02	D6-6	15	ND	ND	ND	ND
53	72/M	I	UM (100)	3	V3-48*01	J5*02	D2-8	21	53.5	0	44	0
54	63/M	IV	M (92.0)	4	V4-34*01	J1*01	D2-21	6	ND	ND	ND	ND
55	38/F	0	M (97.8)	2	V2-5*10	J4*02	D2-21	14	0	0	0	0
56	82/M	IV	UM (100)	1	V1-69*01	J6*02	D3-3	23	0	57	0	0
57	49/F	I	UM (100)	1	V1-2*02	J4*02	D6-19	13	0.4	9.8	ND	0
58	57/M	0	UM (100)	1	V1-69*13	J6*02	D3-3	23	0	0	0	0
59	79/M	I	M (95.0)	3	V3-74*01	J6*13	D6-13	18	70	0	11.2	0
60	82/F	I	M (92.7)	3	V3-33*01	J3*02	D2-8	13	ND	ND	ND	ND
61	69/M	I	M (97.5)	2	V2-5*10	J4*01	D2-21	14	52.4	0	9.4	0
62	68/M	II	M (86.5)	2	V2-5*10	J3*01	D6-19	18	ND	ND	ND	ND
63	70/M	ND	M (93.5)	1	V1-69*13	J6*02	D3-10	27	ND	ND	ND	ND
64	74/M	ND	UM (100)	1	V1-69*02	J4*02	D3-3	18	ND	ND	ND	ND
65	72/F	0	M (93.4)	3	V3-30*04	J4*02	D3-22	21	2.6	10	2.1	ND
66	87/F	0	M (96.8)	3	V3-21*02	J6*02	D1-26	9	4.3	0.5	3.6	1.6
67	75/M	IV	UM (99.5)	3	V3-21*01	J6*02	D3-3	11	ND	6.5	ND	ND
68	69/M	0	UM (98.5)	3	V3-66*01	J1*01	D1-26	12	52.4	1.4	0.99	9.4
69	55/F	IV	M (92.7)	3	V3-23*01	J4*02	D2-2	19	4.1	1.0	1.8	ND
70	67/M	0	M (85.5)	3	V3-48*03	J3*02	D1-14	11	ND	ND	ND	ND
71	36/F	ND	M (89.0)	1	V1-3*01	J3*02	D5-24	19	73.3	0	1.9	0
72	45/M	I	M (93.8)	4	V4-59*01	J4*02	D3-22	13	0	0	0	0
73	79/F	II	M (92.2)	4	V4-61*02	J3*02	D5-18	15	3.3	0.9	3.1	5.0

Abbreviations: X1 = 13q14 monoallelic deletion; X2 = 13q14 biallelic deletion; FISH = fluorescence in situ hybridization; HCDR3 = heavy complementary-determining region 3; M = mutated; MS = mutational status; ND = no data; UM = unmutated.

a higher representation of the IGHV3 family followed by IGHV1 and IGHV4. Interestingly, this distribution was different from patients with CLL from Brazil,[22] Uruguay,[23] and Asia,[16,37-39] who showed IGHV3 > IGHV4 > IGHV1. Differences found among Latin American countries may reflect variations in the genetic background of their populations and/or differences in environmental factors. Thus, the contribution of European, Amerindian, and African ancestries in the Argentinian population is 78%, 19.5%, and 2.5%, respectively,[40] which shows a similar proportion of European parental population (84.1%) but an inverted proportion of Amerindian and African contributions (5.6% and 10.4%, respectively) than Uruguay.[41] The genetic structure of the Brazilian population is markedly different, which revealed a high degree of admixture among individuals from different ethnic origins with a broad genotypic variation.[42] Furthermore, the low representation of IGHV2 and IGHV7 gene families and the lack of expression of IGHV5 and IGHV6 in our

Figure 1 (A) Immunoglobulin Heavy Chain Variable (*IGHV*) Family Usage Profile of Patients With Mutated (M) Chronic Lymphocytic Leukemia (CLL) and Patients With Unmutated CLL (UM-CLL). (B) *IGHV* Gene Segment Usage Profile in Patients With M-CLL and Patients With UM-CLL



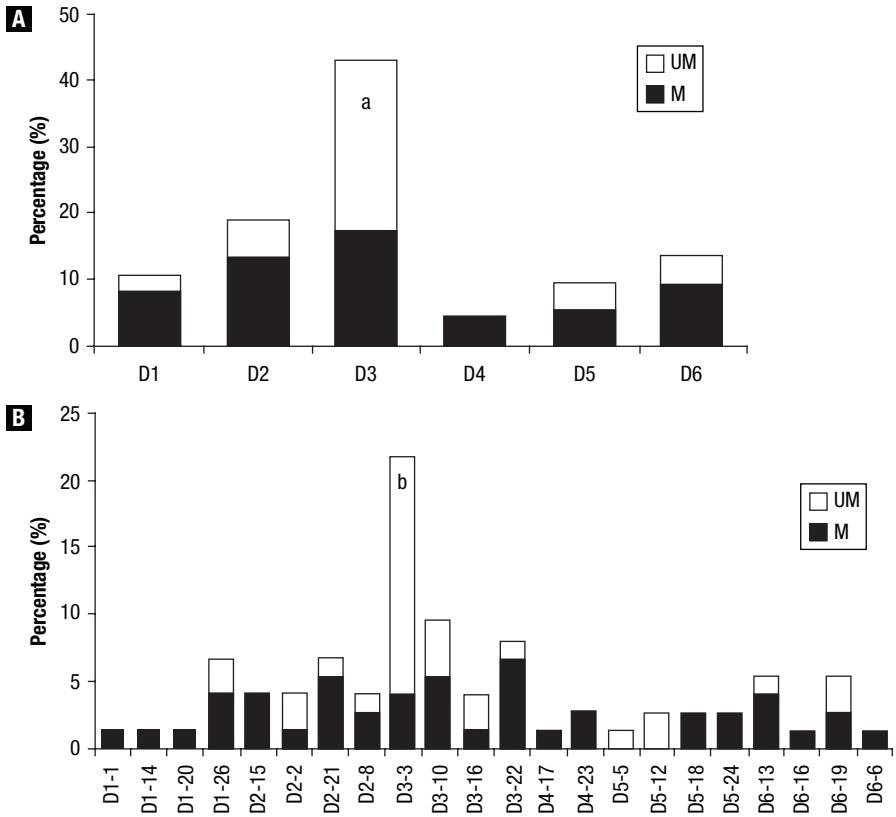
[a]Significant differences in the mutational status between IGHV1 family and IGHV3 ($P = .0039$), IGHV4 families ($P = .0095$), and the entire series ($P = .0016$).

series were also observed in other studies, regardless of their ethnic origin. Even though we would have liked to analyze our results based on the origin of the patients, it was not feasible due to the small size of our series and the scarce information about the individual ethnic origin of the patients included in this study.

With regard to the *IGHV* gene usage, the comparison of our results with data from different geographic regions showed overexpression of *IGHV4-59*, *IGHV3-72,* and *IGVH2-5,* and showed underrepresentation of *IGHV4-34* (Figure 4). The *IGHV4-59* gene had only a comparable expression (6.8%) to that observed in Brazilian[22] (8.1%) and Chinese[38] (10.8%) patients with CLL but a higher frequency than series of Western countries.[10,13,14,18,33] Similarly, results of interest the overexpression of *IGVH2-5* (6.8%) in our series, segment that was found underrepresented in all series except in a small cohort from France (10.3%).[43] In reference to *IGHV3-72*, although it has been found related to highly stable and indolent disease,[44] this association was not confirmed in our series, in which 2 of 3 patients overexpressing this gene required treatment after a follow-up of 48 and 108 months, respectively. In contrast, *IGHV4-34* (2.7%) was underrepresented in Argentinian patients compared with published series from Europe, Asia, and United States.[13,14,16,33,38,45] Low frequencies were also observed in a reduced cohort from Italy (3.3%)[46] and in Iranian patients (5.7%).[47] These differences in *IGVH* gene usage in patients geographically distant suggest selection and clonal expansion of leukemic B cells by participation of specific antigenic stimuli that would influence the pathogenesis and clinicobiologic features of the disease.

In reference to the remaining genes, as previously published, *IGHV3-23*[10,13] was the most frequently used segment, followed by *IGHV1-69*.[11,13,14,33] Low frequency of *IGHV3-23* (1.6%) was only observed in Ukraine patients.[48] This gene is constantly absent from clusters of stereotyped B-cell receptors and has a mutational profile often consistent with superantigen binding.[49] Expression of the *IGHV3-23* gene marks a CLL subset with distinct clinicobiologic features and is considered an independent negative prognosticator for M-CLL.[49] In context with these findings, at the time of this study, 5 (62.5%) of 8 of our patients with *IGHV3-23* rearrangement showed progressive disease, 3 of them required treatment at diagno-

Figure 2 (A) *IGHD* Family Usage of Patients With Mutated (M) Chronic Lymphocytic Leukemia (CLL) (M-CLL) and Patients With Unmutated (UM) CLL (UM-CLL). (B) *IGHD* Gene Segment Usage Profile in Patients With M-CLL and Patients With UM-CLL



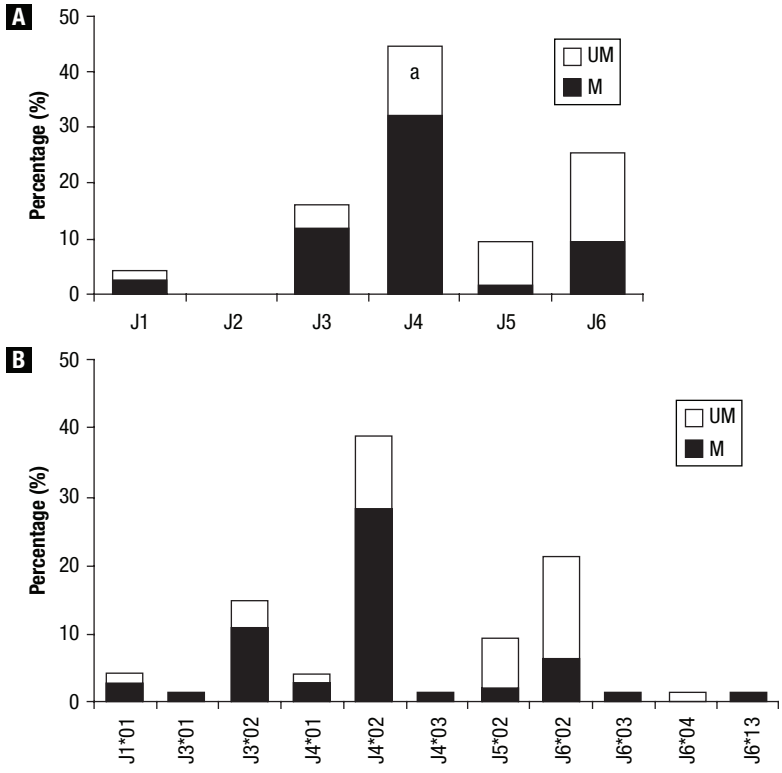
[a]Significant differences in the mutational status between patients with CLL expressing the IGHD3 family and the entire series ($P = .0097$).
[b]Significant differences in the frequency of patients with UM-CLL expressing *IGHD3-3* gene with respect to the whole series ($P = .0005$).

sis. With regard to *IGHV1-69*, it was the most frequently used gene of the IGHV1 family, exhibiting in almost all cases a germline profile.[50] The highest usage of *IGHV1-69* has been reported in Ukrainian (21.7%)[48] and Spanish (21.4%)[7] CLL cases. Our data as well as those from Brazil[22] and Uruguay[23] are higher than frequencies observed in Asian population[16,17,38,51] but lower than most of European[11,13,14] and US series[11,33] (Supplementary material, Table S1). Several groups have demonstrated that *IGHV1-69* rearrangements in CLL display characteristic features, such as biased use of certain *IGHD* and *IGHJ* genes, and a significantly longer average length of the HCDR3 region compared with other CLL cases.[10,50] We could confirm the association of *IGHV1-69* with *IGHD3-3*, *IGHD3-10*, *IGHD2-2,* and *IGHJ6* genes reported by other investigators[10,11,50] and also the presence of longer HCDR3 regions in these cases compared with those displaying *IGHV3-7*, *IGHV3-72, IGHV2-5,* and *IGHV3-23* rearrangements. Two patients expressing *IGHV1-69* showed stereotyped receptors (Table 2).

It is interesting to point out that our series showed a 5.4% frequency for the *IGHV3-21* gene; only 1 patient (1/4 [25%]) (Table 2) had stereotyped B-cell receptor that belonged to subset #2. Abramenko et al[48] showed a similar frequency of this gene (5.8%), with a comparable proportion of stereotyped B-cell receptor (18.2%) in Ukrainian patients with CLL. Different studies demonstrated a high frequency of *IGHV3-21* in a Scandinavian[19,52] CLL population and lower frequencies in larger studies from the United States and Mediterranean region.[11,18,33] Recently, this gene was commonly observed in mestizo Venezuelans in whom they account for 22.2% of all genes,[24] which supports the importance of the genetic background in determining the occurrence of different rearrangements in distinct geographic and/or ethnic groups. Interestingly, mantle cell lymphomas have a remarkable *IGHV* gene repertoire, with *IGHV3-21* being one of the most frequently overexpressed, particularly in patients with UM state, which reveals different immune pathways to lymphoma development, probably through selection of different antigenic stimuli than those in CLL.[53]

As shown, the use of specific *IGHV* genes linked to the *IGHV* M status and the presence of stereotyped B-cell receptor structures has been related to the biologic and clinical characteristic of this disease. This aspect has been much less explored for *IGHD* and *IGVJ* genes, which may play a role in the development of CLL, at least in a subset

Figure 3 (A) *IGHJ* Family Usage Profile of Patients With Mutated (M) Chronic Lymphocytic Leukemia (CLL) (M-CLL) and Patients With Unmutated (UM) CLL (UM-CLL). (B) *IGHJ* Segment Usage Profile in Patients With M-CLL and Patients With UM-CLL



[a]Significant differences in the frequency of patients with M expressing *IGHJ4* segment compared with the whole series ($P = .033$).

Table 2 HCDR3 Sequences of Argentinian Patients With CLL and Stereotyped Receptors

Case No.	Rearrangement			Mutational Status	HCDR3 Sequence	Stamatopoulos, Murray, Bomben Subsets	MAS	Messmer Subsets	MAS
	IGHV Gene	*IGHD* Gene	*IGHJ* Gene						
14	V1-3*01	D6-19	J4*02	UM	EQWLVLASFDY	1	64	—	—
15	V3-30*03	D1-20	J3*02	M	NNWNDFQDASDI	N6	70	26	58
16	V1-3*03	D1-26	J6*02	UM	MYSGSYYYYYYGMDV	—	—	27	73
18	V1-69*01	D3-3	J6*02	UM	PKDSYDFWSGYHVLYYYYGMDV	7	62	9	68
26	V3-30*03	D3-3	J6*02	UM	ADLNADDFWSGYHYYYYYGMDV	7	62	64	63
59	V1-69*13	D3-3	J6*02	UM	DPTGDFWSGYYPNYYYYGMDV	7	71	—	—
66	V3-21*01	D1-26	J6*02	M	TRDANGMDV	2	82	2	88

Abbreviations: CLL = chronic lymphocytic leukemia; HCDR3 = heavy complementary-determining region 3; IGHV = immunoglobulin heavy chain variable; M = mutated; MAS = mean alignment score; UM = unmutated.
MAS was the mean value of all the pairwise alignment scores as determined by ClustalW (Ref. 32).
Subsets 1, 2, and 7 identified Stamatopoulos et al[13] and Murray et al,[30] and N6 proposed by Bomben et al,[14] subsets 2, 9, 26, 27, and 64, identified by Messmer et al.[31]

of patients. In supporting this notion, Mauerer et al[11] found a shorter time to treatment in patients who used IGHD2 and D3, and Tschumper et al[54] had similar results in a subset of patients expressing the *IGHD3-3* gene in reading frame 2, an association not found for IGHJ genes. In concordance, 3 of 7 patients with stereotyped HCDR3 from our series showed *IGHD3-3*, 2 of them with a short time to treatment.

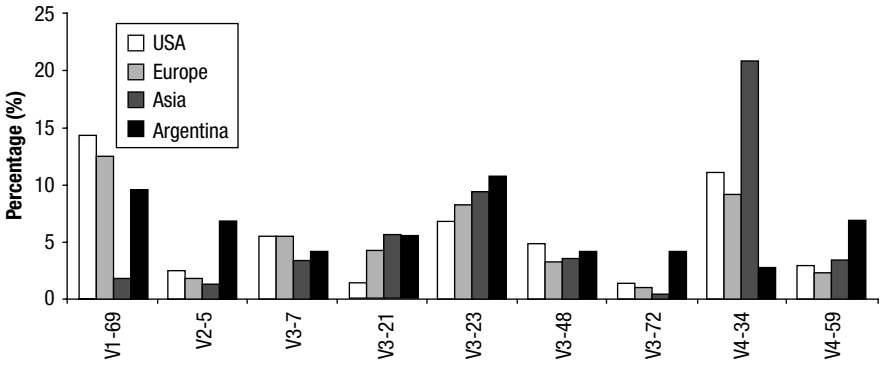
As for expressions of stereotyped B-cell receptors, they were found in 9.5% of our cohort, which suggests that an antigen-driven process may have occurred in these cases. This percentage was lower than

Table 3 IGHV Family Usage in Patients With CLL From Different Geographic Regions

	Total No. Sequences	IGHV Family (%)							Reference No.
		IGHV1	IGHV2	IGHV3	IGHV4	IGHV5	IGHV6	IGHV7	
United States	1188	26.9	2.0	48.0	20.3	1.0	0.7	0.3	36
Sweden	407	28.0	2.0	47.0	19.5	<2	<2	<2	52
Italy	1456	22.3	2.1	50.8	21.4	2.3	0.6	0.5	14, 46
Spain	56	39.3	3.6	35.7	17.8	1.8	0	1.8	7
France	39	23.1	10.3	41.0	15.4	7.7	2.5	0	43
Mediterranean	927	24.3	2.8	46.1	23.4	2.4	0.8	0.2	13
England	243	25.5	5.7	49.3	15.6	2.5	1.2	0	6, 10
Japan	155	9.7	3.9	49.7	33.0	2.6	1.3	0	16, 37, 51
China	111	9.0	2.7	46.8	37.8	2.7	0	0.9	17, 38
Islamic Republic of Iran	87	18.4	0	56.4	20.7	1.1	3.4	0	47
Brazil	37	10.8	5.4	37.8	35.2	8.1	2.7	0	22
Uruguay	80	17.5	2.5	52.5	22.5	1.3	1.3	2.5	23
Venezuela	87	25.3	0	48.3	21.8	4.6	0	0	24
Argentina	74	23	6.8	52.7	16.2	0	0	1.4	Present study

Abbreviations: CLL = chronic lymphocytic leukemia; IGHV = immunoglobulin heavy chain variable.

Figure 4 *IGHV* Gene Segment Usage in Patients With Chronic Lymphocytic Leukemia From Different Geographic Regions



that reported in previously published series[13,14,30,33] but comparable with those observed by Bianchi et al[23] and Tobin et al[52] (12.5% each). As found in other studies,[13,14,52] most of stereotyped receptors were observed in patients with UM-CLL, especially in sequences with a 100% IGHV identity[30] and in the IGHV1 family rather than IGHV3.[13] In addition, significant associations between different stereotyped HCDR3 sequences and genomic abnormalities were described. Particularly, subset #2 (*IGVH3-21*) showed a strong prevalence of del(13q14),[55,56] an alteration that was also observed in a lower frequency in nonsubset #2 patients with *IGVH3-21*.[55] In our series, the only patient from subset #2 did not show del(13q14), but this alteration was present in the 2 cases from nonsubset #2 evaluated by FISH, both in initial Rai stages. Moreover, Maura et al[56] found a high prevalence of unfavorable deletions, particularly deletion of chromosome 17p13, in subset #2 patients and a low incidence of genomic aberrations in subset #4. With regard to the clinical outcome, subset #1, the most frequent subset in patients with CLL,[15] mostly UM, is associated with poor prognosis,[13,14] as well as subset #7.[13] Two of our 3 patients who belonged in subset #7 showed progressive disease, and one of them (case 16) had autoimmune hemolytic anemia. In this aspect, Maura et al[57] found subset #3 as the most frequently associated with autoimmune hemolytic anemia, which suggests that this complication was principally related to the mutational status and/or specific HCDR3 sequences. It is interesting to point out the aggressive clinical course complicated by severe recurrent infections, Richter transformation, or the occurrence of second solid

tumors observed in stereotyped *IGVH4-39*.[58] We did not have patients expressing this gene, and our only patient (case 27) with Richter transformation showed *IGVH4-59* expression and UM status.

More recently, it has been suggested that a restricted set of some common antigens reactive with CLL B cell receptors are important for the development and expansion of this disease. Among them, a nonmuscle myosin heavy chain IIA, has been identified as an autoantigen that is recognized by a specific CLL subset and became exposed on the cell surface during apoptosis.[59] Interestingly, this subset of patients has been associated with poor clinical outcome, which suggests that the prognosis of patients with CLL would reflect the antigen binding.[60] These findings indicate the importance of the study of stereotypy in CLL for improving patient stratification toward personalized therapeutic applications.

Conclusion

We have studied for the first time *IGHV-D-J* gene rearrangements in Argentinian patients with CLL. Our results were coincident with those reported in Western populations and also showed interesting similarities and differences with respect to published series from the Latin American region. The analysis of a large number of patients will contribute to clarify whether these results reflect variations in the genetic background and/or differences in environmental factors of this geographic region.

Clinical Practice Points

- The mutational status of the *IGHV* genes represent one of the best prognostic markers in CLL and defines 2 disease subgroups: M-CLL, with a more favorable clinical course, and UM-CLL, which is associated with a poor outcome.
- The analysis of genomic abnormalities by using FISH allowed the identification of distinct cytogenetic risk groups in which patients with del(13q14) as a single alteration have a better outcome, whereas patients with deletions of chromosome 11q22 or 17p13 show the shortest median survival, and cases with trisomy 12 have an intermediate prognosis.
- *IGHV* gene repertoire analysis in CLL has demonstrated biases both at the IGH subgroup level and at the specific gene level, with ethnic and geographic variations that may reflect differences in the genetic background and/or in environmental factors.
- This is, to our knowledge, the first study of *IGHV-D-J* gene rearrangements and mutational status in Argentinian patients with CLL. Our results showed that 43 (58.9%) cases belonged to M-CLL, whereas the remaining 30 (41.1%) were classified as UM-CLL.
- Del(13q14) as a single alteration was more frequently observed in patients with M-CLL (48%) vs. patients with UM-CLL (24%). When the M-CLL group was analyzed, a significant increase in the proportion of cases with del(13q14) compared with the frequencies of +12 and deletions of chromosomes 11q and 17p was found ($P = .003$).
- IGVH family distribution in our cohort showed a higher representation of IGHV3 followed by IGHV1 and IGHV4, which resembled that observed in Western countries. This distribution was different from Brazilian and Uruguayan series that showed IGHV3 > IGHV4 > IGHV1. The analysis of *IGHV* genes showed overrepresentation of *IGHV4-59*, *IGHV3-72,* and *IGHV2-5,* and underusage of *IGHV4-34* in our patients with CLL compared with published data.
- Expression of the *IGHV3-23* gene is considered an independent negative prognosticator for M-CLL. In coincidence, 5 (62.5%) of 8 patients from our series expressing this gene showed progressive disease, 3 of the patients required treatment at diagnosis. On the contrary, the association between highly stable and/or indolent disease and the *IGHV3-72* gene was not observed in our series. Although the number of cases is small, 2 of 3 patients expressing *IGHV3-72* gene rearrangement required treatment after a follow-up of 48 and 108 months, respectively.
- Our results showed that Argentinian patients with CLL displayed an *IGHV* gene usage that resembles that observed in Western countries and that exhibit interesting similarities and differences with respect to published series from other Latin American populations, which reflect variations in the genetic background and/or differences in environmental factors. Further studies may clarify this point.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:http://dx.doi.org/10.1016/j.clml.2013.02.019.

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Table S1 Comparison of IGHV Gene Usage in CLL Patients from Different Geographic Regions

Reference	Total of Sequences	IGHV Member - N° Cases (%)										
		1-2	1-69	3-7	3-21	3-23	3-72	4-34	3-48	3-43	4-59	2-5
United States												
1	375	18 (4.8)	68 (18)	16 (4.3)	6 (1.6)	26 (7)	2 (0.5)	26 (7)	16 (4.3)	0	0	11 (3)
2	172	9 (5.2)	21 (12.2)	9 (5.2)	0	11 (6.4)	6 (3.5)	27 (15.7)	15 (8.7)	0	10 (5.8)	6 (3.5)
3	172	11 (6.4)	18 (10.5)	14 (8.2)	2 (1.2)	11 (6.4)	2 (1.1)	25 (14.5)	5 (2.9)	0	8 (4.7)	ND
4	41	1 (2.4)	2 (4.9)	2 (4.9)	2 (4.9)	4 (9.8)	0	6 (14.6)	0	0	2 (4.9)	1 (2.4)
Europe												
5	407	17 (4)	58 (14)	14 (3)	35 (9)	21 (5)	NI	28 (7)	NI	NI	NI	NI
6	25	2 (8.0)	1 (4.0)	0	1 (4.0)	4 (16.0)	0	3 (12.0)	0	0	0	2 (8)
7	1426	60 (4.2)	169 (11.9)	83 (5.8)	58 (4.1)	134 (9.4)	27 (1.9)	128 (9.0)	54 (3.8)	0	46 (3.2)	21 (1.5)
8	30	4 (13.3)	1 (3.3)	2 (6.6)	1 (3.3)	0	0	1 (3.3)	2 (6.7)	0	0	0
9	56	1 (1.8)	12 (21.4)	1 (1.8)	1 (1.8)	3 (5.4)	2 (3.6)	4 (7.1)	2 (3.6)	1 (1.8)	1 (1.8)	1 (1.8)
10	39	2 (5.1)	4 (10.2)	1 (2.6)	1 (2.6)	5 (12.8)	0	4 (10.3)	0	0	0	4 (10.8)
11	927	38 (4.1)	115 (12.6)	60 (6.6)	32 (3.5)	82 (9.0)	7 (0.8)	95 (10.4)	33 (3.6)	4 (0.43)	22 (2.4)	21 (4)
12	553	22 (4.2)	59 (10.7)	30 (5.4)	17 (3.0)	51 (9.2)	0	59 (10.7)	11 (2.2)	NI	11 (2.0)	17 (3.1)
13	84	6 (7.1)	10 (11.9)	6 (7.1)	2 (2.4)	10 (11.9)	0	10 (11.9)	4 (4.8)	0	1 (1.2)	1 (1.2)
14	159	10 (6.3)	18 (11.3)	8 (5.0)	9 (5.7)	13 (8.2)	3 (1.9)	11 (6.9)	9 (5.6)	1 (0.6)	1 (0.6)	7 (4.4)
15	189	10 (5.3)	41 (21.7)	9 (4.8)	11 (5.8)	3 (1.6)	0	14 (7.4)	8 (4.2)	0	7 (3.7)	0
Asia												
16	43	NI	1 (2.3)	NI	3 (7)	6 (14)	NI	9 (20.9)	NI	3 (7.0)	NI	NI
17	80	1 (1.25)	1 (1.25)	4 (5)	6 (7.5)	7 (8.75)	0	22 (27.5)	3 (3.75)	1	0	0
18	65	0	1 (1.5)	3 (4.6)	2 (3)	5 (7.7)	0	8 (12.3)	1 (1.5)	0	7 (10.8)	3 (4.2)
19	46	1 (2.2)	1 (2.2)	1 (2.2)	2 (4.3)	4 (8.7)	1	10 (21.7)	4 (8.7)	0	1	0
20	87	3 (3.4)	5 (5.7)	11 (12.6)	0	4 (4.6)	0	5 (5.7)	8 (9.2)	0	0	0
South America												
21	37	0	2 (5.4)	2 (5.4)	0	4 (10.8)	1	3 (8.1)	0	0	3 (8.1)	0
22	80	3 (3.75)	6 (7.5)	10 (12.5)	1 (1.3)	9 (11.3)	1	5 (7.5)	4 (5.0)	0	1 (1.25)	0
23	87	4 (4.6)	13 (14.9)	7 (8)	8 (9.2)	13 (14.6)	0	9 (10.3)	3 (3.4)	0	2 (2.3)	0
Present study	**74**	**2 (2.7)**	**7 (9.5)**	**3 (4.1)**	**4 (5.4)**	**8 (10.8)**	**3 (4.1)**	**2 (2.7)**	**3 (4.1)**	**1 (1.3)**	**5 (6.8)**	**5 (6.8)**

Abbreviation: NI = not identified.

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