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IGHV3-21 GENE EXPRESSION IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IN UKRAINE

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The aim **of the study was to evaluate the frequency of *IGHV3-21* gene usage and its clinical significance for patients with B-cell chronic lymphocytic leukemia (CLL) in Ukraine.** ***Patients and Methods:*** **Immunoglobulin variable heavy chain (*IGHV*) gene repertoire was studied in 189 CLL patients using reverse transcribed polymerase chain reaction and direct sequence of amplified products.** ***Results:*** ***IGHV3-21*** **gene expression was found in 11 cases (5.8%), and its frequency was intermediate between Scandinavian (11.7%) and Mediterranean CLL (2.9%) cohorts. The most of cases (9 of 11) belonged to subset with heterogeneous HCDR3 (heteroHCDR3 subset), and only 2 cases – to subset with classical short ARDANGMDV motif (homHCDR3 subset). Six *IGHV3-21* cases were mutated and 5 cases were unmutated. All unmutated cases (all were from heteroHCDR3 subset) had similarity of their HCDR3s with previously published sequences. The differences in overall (OS), progression-free (PFS) and treatment-free survival (TFS) for *IGHV3-21* positive patients in comparison with CLL patients expressing the other *IGHV* genes were statistically insignificant. These survival parameters were comparable also for CLL patients with mutated *IGHV3-21* gene usage and expression the others mutated *IGHV* genes. But remarkable feature of *IGHV3-21* expressing patients was high incidence of solid tumors. They have developed in 4 *IGHV3-21* positive cases (36.4%) and in 10 cases with expression of the others IGHV genes (5.6%, $p = 0.0002$). Furthermore, in small group of 6 patients with mutated *IGHV3-21* gene expression, 3 patients had solid tumors and one underwent Richter transformation. Unmutated *IGHV3-21* gene expressed patients had worse OS and PFS in comparison with CLL patients that expressed the others unmutated *IGHV* genes.** ***Conclusion:*** **Presented data are in agrement with the opinion about negative prognostic significance of *IGHV3-21* gene expression regardless its mutation status. *IGHV3-21* expression was associated with development of secondary solid tumors. Revealed high level of homology in heteroHDR3s subset might suggest about possible antigenic influence also, in addition to homHCDR3 subset that was proposed earlier**

Key Words: **chronic lymphocytic leukemia, immunoglobulin variable heavy chain gene, *IGHV3-21,* prognostic factor.**

In recent years, one of the main focuses in investigation of B-cell chronic lymphocytic leukemia (B-CLL) pathogenesis consists in the study of antigenic influence as possible initial stimulus for development of malignant lymphoid clone [1]. Now B-CLL is considered as disease of immunologically competent B-lymphocytes that undergo antigen selection [2, 3]. Investigation of variable (*V*), diversity (*D*), and joint (*J*) immunoglobulin (IG) heavy (*H*) chain genes are used to construct of B-cell antigen receptor (BCR) revealed nonstochastic distribution that was differed from expected random assortment and from normal peripheral blood B-lymphocytes. Besides this, more than 20% of B-CLL patients from different geographic regions have very similar BCR structures with high degree of homology in third complementarity determining regions (CDR3) of both heavy and light (L) immunoglobulin chains [4]. As the probability of specific *IGHV*/*IGHD*/*IGHJ* rearrangement together with *IGLV*/*IGLJ* rearrangement is 1 in 1.32 million, the antigenic influence on CLL cell clone seems important.

Now 48 B-CLL subsets with stereotyped HCDR3 are described [4]. The most frequently homologous HCDR3 are found among cases with *IGHV1-69* and *IGHV3-21* gene usage. CLL cases with *IGHV3-21* gene usage now are considered as distinct entity with restricted immunoglobulin gene features and poor prognosis [5]. Overrepresentation of *IGHV3-21* gene was first revealed in Scandinavian cohort of CLL patients (11.7%), and the most of presented *IGHV3-21* cases had HCDR3 conservative amino acid sequence consisted of a Ala-Arg-Asp-Ala-Asn-Gly-Met-Asp-Val (ARDANGMDV) motif (CLL subset #2) [6, 7]. It is well known that the absence of somatic hypermutations in the *IGHV* genes is negative prognostic factor in CLL [8, 9], but mutated *IGHV3-21* cases (most of them with ARDANGMDV motif) had worse overall survival, which was significantly different from the remaining mutated CLL cases and similar to the unmutated group [7]. These data were confirmed in studies of British and Belgian CLL patients [10, 11]. P. Ghia et al. [5] have shown that worse prognosis was typical first of all for homogeneous HCDR3 subset (homHCDR3) while *IGHV3-21* cases with heterogeneous HCDR3 (heteroHCDR3) had variable clinical course. They also reported that *IGHV3-21* gene widely represented in Northern Europe was found only in 2.9% CLL patients from the Mediterranean area (France, Greece, Italy, Spain). The comprehensive study of 1076 CLL cases from Northern, Central and Southern Italy confirmed

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Abbreviations used: BCR – B-cell antigen receptor; CDR3 – third complementarity determining region; CLL – chronic lymphocytic leukemia; FACS – fluorescence-activated cell sorting analysis; IG – immunoglobulin; IGHD – immunoglobulin diversity heavy chain; IGHJ – immunoglobulin joint heavy chain; IGHV – immunoglobulin variable heavy chain; IGLV – immunoglobulin variable light chain; IGLJ – immunoglobulin joint light chain; OS – overall survival; PCR – polymerase chain reaction; PFS – progression-free survival; TFS – treatment-free survival.

relatively low frequency of *IGHV3-21* gene usage in this region (3.4%) and worse prognosis for homogeneous HCDR3 subset, but also revealed nonrandom distribution of homHCDR3 and heteroHCDR3 subsets: the first was found almost exclusively in Northern Italy (16 of 18 cases) and the last (19 cases) was uniformly distributed throughout Italy [12]. The assumption about different antigenic influence in certain geographic regions was proposed.

The aim of this work was to study the frequency of *IGHV3-21* gene usage and its clinical significance in Ukrainian CLL cohort.

MATERIALS AND METHODS

A total of 189 CLL patients from different regions of Ukraine, mainly from its Central part, were studied for *IGHV* gene usage and mutation status (Table 1). The diagnosis of CLL was based on clinical history, lymphocyte morphology, and immunophenotypic criteria. Stage of disease was established according to classifications of Rai [13], Binet [14] and International Workshop on Chronic Lymphocytic Leukemia [15].

Table 1. Observed CLL patients, region of Ukraine, number of patients

Western Ukraine		Central Ukraine		Southern Ukraine		Eastern Ukraine	
L'viv	4	Kiyv	59	Odessa	2	Dnepropetrovsk	9
Luzk	2	Poltava	67	Nikolaev	1	Donesk	9
Rivne	2	Sumy	4	Crimea	3	Lugansk	5
Ternopyl	1	Cherkassy	8			Charkiv	1
Chmelnizkiy	2						
Zhitomir	6						
Chernovzy	1						
Vinniza	3						
Total cases	21	Total	138	Total	6	Total	24
IGHV3-21 gene positive cases	0		9		0		2

Total RNA was extracted from peripheral blood and/or bone marrow cells using guanidine isotiocyanate-phenol-chlorophorm extraction according to Chomczynski [16]. 1–5 μg of the total cellular RNA was reverse transcribed to cDNA using MuLV reverse transcriptase enzyme (Applied Biosystem, Branchburg, New Jersey, USA) and random hexamers as primers. cDNA was amplified in a single multiplexed PCR reaction consisting of six *IGHV* framework 1 primers combined with *IGHJ* consensus primer as designed by the BIOMED-2 consortium [17]. All reactions were carried out in final volume of 50 μl with 10 pmol of each primer, 200 nM dNTPs, 1 U AmpliTaq Gold and 10 x PCR buffer II (Applied Biosystems, Branchburg, New Jersey, USA). The cycling conditions were as follows: preactivation at 94 °C for 7 min; 35 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 45 s; a final cycle of 10 min at 72 °C.

PCR products were analyzed on a 1.5% agarose gel and visualized with ethidium bromide staining. Products were spin column purified using PCR purification kit (Promega, Madison, WI) and sequenced directly using forward and reverse primers in an automated DNA sequencer ABI-310 (Applied Biosystem, Foster City, CA) using BigDye Terminator Cycle Sequencing Reaction Kit (Perkin Elmer, Foster City, CA). The closest germline *IGHV* gene for each B-CLL IGHV sequence was assigned using current databases IMGT (International Immunogenetics Database) [18], IgBlast (National Center for Biotechnology Information, Bethesda, MD), and JoinSolver [19]. *IGHV* gene sequences deviating more than 2% from the corresponding germline gene were defined as mutated. For *IGHD* gene determination, a requirement of a minimum of 7 matching nucleotides was used. The length of the HCDR3 was calculated followed established IMGT criteria.

In some cases with homologous *IGHV* sequences we amplified immunoglobulin variable light kappa or lambda chains (*IGKV* and *IGLV*) according to the BIOMED-2 protocol [17].

Statistics was performed using the SPSS 13.0 software package (SPSS, Chicago, IL).

RESULTS

IGHV gene usage and mutation status. Productive *IGHV-D-J* rearrangements were sequenced in all 189 CLL patients. Using the 98% cut off for homology to germ line, 55 (29.1%) cases were classified as mutated with a mean mutation frequency of 7.3% (range, 3.6–11.6%). The remaining 134 rearrangements (70.9%) showed <2% mutations and were classified as unmutated.

The top 10 most used *IGHV* genes in this CLL cohort were *IGHV1-69* (41 cases; 21.7%), *IGHV4-34* (14; 7.4%), *IGHV3-21* and *IGHV3-33* (each 11 cases; 5.8%), *IGHV1-02* (10; 5.3%), *IGHV3-07* (9; 4.8%), *IGHV3-48* and *IGHV3-09* (each 8 cases; 4.2%), followed by *IGHV3-11*, *IGHV4-59*, and *IGHV3-30* (each 7 cases, 3.7%). There were prevalence of unmutated cases and increased frequency of *IGHV1-69* and *IGHV3-09* gene positive cases in our cohort in comparison with previously published data [20–22]. The frequency of *IGHV3-21* gene usage (5.8%) was intermediate between Scandinavian (11.7%) and Mediterranean CLL (2.9%) cohorts. There were no differences in frequency of *IGHV3-21* gene usage in patients from different regions of Ukraine probably because of the prevalence of observed patients from the Central part.

Six (54.5%) *IGHV3-21* positive cases were mutated with a mean mutation frequency of 4.3% (range, 2.7–6.8%), and 4 cases had low level of *IGHV* mutations (between 2 and 4%) that is typical for mutated *IGHV3-21* gene used cases [10]. *IGHV3-21* gene was on the third position within the mutated subset (10.9%), where *IGHV4-34* followed by *IGHV3-07* were the most represented *IGHV* genes. In the unmutated subset *IGHV3-21* gene (5 cases) shared 5-9 positions with *IGHV3-09*, *IGHV3-30*, *IGHV3-48*, and *IGHV4-59* genes by the frequency of usage (Table 2).

Table 2. *IGHV* gene usage in all mutated and unmutated cases

Rank	Mutated IGHV gene	% (n cases)	Unmutated IGHV gene	% (n cases)
1	IGHV4-34	20 (11)	IGHV1-69	29.9 (40)
2	IGHV3-07	14.5 (8)	IGHV1-02	6.7 (9)
3	IGHV3-21	10.9 (6)	IGHV3-33	6.7 (9)
4	IGHV3-09	5.5 (3)	IGHV3-11	4.8 (6)
5	IGHV3-23	5.5 (3)	IGHV3-09	3.7 (5)
6	IGHV3-48	5.5 (3)	IGHV3-21	3.7 (5)
7	IGHV3-53	5.5 (3)	IGHV3-30	3.7 (5)
8	IGHV3-15	3.6 (2)	IGHV3-48	3.7 (5)
9	IGHV4-59	3.6 (2)	IGHV4-59	3.7 (5)
10	IGHV3-30	3.6 (2)	IGHV4-39	3.0 (4)

Only two (18.2%) *IGHV3-21* cases (both mutated) in our CLL group belonged to subset #2 with homogeneous HCDR3 (EF407845 and EF407838). Their HCDR3 sequences were identical, and their LCDR3 differed only by one amino acid, to the sequences published early (Table 3). Nine *IGHV3-21* cases belonged to the so-called heterogeneous HCDR3 subset with unrelated HCDR3 rearrangements, but we found that 3 of them (EF091900, EF091902, and EF407842) had showed homology with heteroHCDR3 *IGHV3-21* cases from another CLL cohorts. Besides this, HCDR3s of two another our heterogeneous *IGHV3-21* cases (EF091901 and EF407825) had some similarity with HCDR3s of previously described *IGHV3-09* and *IGHV1-69* cases respectively (see Table 3). All of these cases were unmutated, so all 5 unmutated *IGHV3-21* cases in our group had similarity of their HCDR3 with another sequences.

Clinical data. *IGHV3-21* gene expression was found in 8 men and 3 women with the mean age 57.7 years at the diagnosis (range, 41–73 years). The mean age and male/female distribution did not differ from CLL patients with the other than *IGHV3-21* gene usage (136 men, 42 women, mean age, 56.9 years).

CD38 expression was studied in 155 patients using by two-color fluorescence-activated cell sorting (FACS) analysis. High level of CD38 expression (> 30%) was found in 6 of 10 (60%) IGHV3-21 positive patients and in 47 of 145 (32.4%) the other CLL patients ($p = 0.07$), and the mean of CLL cells expressing CD38 was higher in *IGHV3-21* subgroup (40.2% vs 22.2%, $p = 0.03$). It is interesting that level of CD38 expression did not differ between mutated and unmutated cases in the whole group of CLL patients (18.8% vs 25.1%; $p = 0.19$), but when *IGHV3-21* positive cases had been excluded, these differences obtained statistical significance (15.2% vs 24.6%; $p = 0.02$). At the same time within IGHV3-21 subgroup number of CD38 expressing CLL cells was similar in mutated and unmutated cases (43.7% vs 36.6%, $p = 0.73$).

Despite of low numbers of *IGHV3-21* cases in our CLL group we found clear differences between mutated and unmutated cases regarding of treatment-free survival (TFS, median 48 months and 7 months, correspondingly, $p = 0.04$), progression-free survival (PFS, median 108 months and 22 months, $p = 0.006$), and overall survival (OS, median did not reach and 43 months correspondingly, $p = 0.02$).

There were no statistically significant differences between mutated *IGHV3-21* cases and mutated cases that expressed the others *IGHV* genes in OS, TFS, and PFS (Table 4). But unmutated *IGHV3-21* expressing cases in comparison with the other unmutated cases were characterized worse OS (median 43 months and 90 months, $p = 0.0008$), and PFS (median 22 months and 48 months, $p = 0.06$), while median TFS curves were similar (7 months and 9 months, $p > 0.05$). When *IGHV3-21* cases were analyzed as a whole group, the differences in OS, PFS, and TFS were insignificant in comparison with the remaining unmutated and the remaining mutated CLL cases as well as both of them.

Table 4. Survival parameters of observed CLL patients

Patients	Median survival curves, months		
	TFS	PFS	OS
All CLL patients, n = 189	12	52	106
Only IGHV3-21 cases, n = 11	34	72	107
CLL patients excluding IGHV3-21 cases, n = 178	12	50	101
Mutated cases			
All mutated cases, n = 55	24	77	Did not reach
Mutated IGHV3-21 cases, n = 6	48	108	Did not reach
Mutated cases excluding IGHV3-21, n = 49	15	77	Did not reach
Unmutated cases			
All inmutated cases, n = 134	9	43	90
Unmutated IGHV3-21 cases, n = 5	7	22	43
Unmutated cases excluding IGHV3-21, n = 129	11	48	90

Remarkable feature of *IGHV3-21* expressing CLL patients was high incidence of solid tumors. They have developed in 4 patients: prostate cancer (180 months before CLL diagnosis; unmutated, heteroHCDR3 subset), colon cancer (simultaneously with CLL diagnosis, mutated, heteroHCDR3 subset), stomach cancer (84 months after CLL diagnosis, mutated, heteroHCDR3 subset), and prostate cancer (228 months after CLL diagnosis, mutated, homoHCDR3 subset). Among 178 CLL patients expressing the other *IGHV* genes, secondary solid tumors were diagnosed only in 10 cases (5.6%, $p = 0.0002$) — one case (2.0%) within mutated subgroup and 9 cases (7.0%) within unmutated subgroup. The frequency of solid tumor's development within *IGHV3-21* used mutated cases was too much higher than among mutated cases expressed the others *IGHV* genes (50% vs 2%; $p < 0.001$). For unmutated cases this difference was insignificant regardless to *IGHV3-21* gene usage. The frequency of Richter transformation did not differ

Table 3. *IGHV3-21* CLL cases with homologous IGHV sequences, (-) indicate homology

N	Case	HCDR3 aa sequences; HCDR3 aa identity between presented paired sequences#	*IGKV/IGLV* gene and K/LCDR3 sequence
1	EF407845*	…AR DMNAMDV	IGLV3-21; QVWDSSSDHPWV
	ITA-1 [4]	…AR -------HCDR3 homology 100%	IGLV3-21; QVWDSGSDHPWV
2	EF407838*	…AR DANGMDV	IGLV3-21; QVWDSSSDHPWV
	P1531 [4]	…AR -------HCDR3 homology 100%	IGLV3-21; QVWDSGSDHPWV
3	EF091900*	…AR DRGVSSSWYLSYYYYMDV	
	Case28 [7]	…AR V--------A-------- HCDR3 homology 90%	
4	EF091902*	…AR NRYTEYCSSTSCHPSYYYYYGMDV	
	Gre6 [5]	…AR D-LLG-------WD ---------HCDR3 homology 73.1%	
5	EF407842*	…AR DSDYDFWSGSWGYYGMDV	IGKV2-30; MQGTHWPPT
	DQ987781*	…AR -G-------Q-------- HCDR3 homology 90%	
6	EF091901* [23]	…AR DGLYDFWSGYGN	
		…AR H-------P-LSPFDY	
7	EF407825*	…AR DGGSLYYDILTGYYSLEYYYGMDV	IGKV1-9; RQLNSYPLFT
	FRA-132 [4]	…AR E-- -R-------- -EY-------	

*GenBank database accession numbers.

significantly between *IGHV3-21* gene used cases (one case, 9.1%) and the other *IGHV* gene used subgroup (10 cases, 5.6%) as well as frequency of autoimmune disorders (9.1% vs 7.9%, $p > 0.05$).

Special attention was devoted to cases with short homogenous HCDR3 sequence. Both of them were diagnosed in A(I) stage, did not need treatment over a long period of time (96 months and 183 months), and had long PFS (120 months and 233 months). However, in one patient disease was complicated by development of prostate cancer, and second patient underwent Richter transformation.

DISCUSSION

In our study *IGHV3-21* gene was found in 11 of 189 productive *IGHV-D-J* rearrangements (5.8%). So, as was mentioned above, the frequency of *IGHV3-21* gene usage in Ukrainian CLL group was intermediate between Scandinavian [6, 7] and Mediterranean cohorts [5] of patients that might have supposed in view of geographical position of Ukraine. But in contrast to others authors the most of our cases belonged to so-called heteroHCDR3 subset (9 of 11) and only two had short ARDANGMDV motif. For example, 21 of 31 *IGHV3-21* Scandinavian cases belonged to the homoHCDR3 subset [7] and the ratio of homo/hetero HCDR3 subsets was approximately equal in Mediterranean study (7/9) [5] and Italian multicenter study (18/19) [12]. Besides this, our two patients with *IGHV3-21* and *IGLV3-21* genes usage from homoHCDR3 subset had had indolent disease for a long time, while the poor prognosis of *IGHV3-21* expressing cases is related namely with homoHCDR3 subset. The differences in the frequency of individual *IGHV* gene usage in different CLL cohort in many respects may be related with basic characteristics of observed patients. Mainly hospital patients with severe course of disease and mostly unmutated *IGHV* gene usage represented our cohort. So, in our group it would rather expect decreasing frequency of some *IGHV* genes (for example, *IGHV3-07*) associated with favorable prognosis than *IGHV3-21* positive cases with assumed bad prognosis. Therefore we supposed that the frequency of *IGHV3-21* cases with homologous HCDR3 was really low in the group of CLL patients from Central part of Ukraine and the influence of hypothetic antigen specific to the common HCDR3 and LCDR3 was not such significant as in Northern European countries. Low frequency of homoHCDR3 subset within *IGHV3-21* expressing cases was also revealed in Southern and Central Italy, and Greek (one of 7 cases) [5].

Concerning to *IGHV3-21* expressing cases with heterogeneous HCDR3 P. Ghia et al. [5] supposed that this subset might reflect normal repertoire of human peripheral blood B-lymphocytes. We observed 9 cases belonged to heteroHCDR3 subset, 4 of them were mutated, and 5 were unmutated. It was interesting that for all 5 unmutated heteroHCDR3 cases we found some similar HCDR3 sequences published earlier. So, it is possible, that expression of *IGHV3-21* gene with heterogeneous HCDR3 is non-stochastic and also reflects possible antigenic stimulation.

We did not reveal statistically significant differences in OS, PFS, and TFS for *IGHV3-21* positive cases in comparison with CLL cases expressing the other *IGHV* genes. These survival parameters of mutated *IGHV3-21* gene used cases were comparable with mutated cases that expressed the others *IGHV* genes. But remarkable feature of mutated *IGHV3-21* cases was high frequency of solid tumors developed before or after CLL diagnosis. Their presence significantly complicated life quality of CLL patients, required operative treatment in all cases, and was direct cause of death of one patient. Thus, in small group of 6 patients with mutated *IGHV3-21* gene expression 3 patients had solid tumors and one underwent Richter transformation. Unmutated *IGHV3-21* gene expressed cases had worse OS and PFS in comparison with unmutated CLL cases that expressed the others genes. These data are in agreeing with the opinion about negative prognostic significance of *IGHV3-21* gene expression regardless its mutation status.

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ЭКСПРЕССИЯ ГЕНА *IGHV3-21* У БОЛЬНЫХ ХРОНИЧЕСКИМ ЛИМФОЛЕЙКОЗОМ В УКРАИНЕ

Цель: **оценить частоту использования гена *IGHV3-21* и его клиническое значение для больных В-клеточным хроническим лимфолейкозом (ХЛЛ) в Украине.** ***Больные и методы исследования:*** **репертуар генов вариабельных участков тяжелых цепей иммуноглобулинов (*IGHV*) изучали у 189 больных с ХЛЛ с помощью полимеразной цепной реакции на базе обратной транскрипции и прямого сиквенса амплифицированных продуктов.** ***Результаты:*** **экспрессия гена *IGHV3-21* выявлена у 11 пациентов (5,8%), что занимает промежуточное положение между Скандинавской (11,7%) и Средиземноморской (2,9%) когортами. Большинство случаев (9 из 11) относились к подгруппе с гетерогенным третьим комплементарным регионом (heteroHCDR3 подгруппа) и только 2 случая — к подгруппе с коротким классическим ARDANGMDV мотивом (homHCDR3-подгруппа). Шесть *IGHV3-21*-позитивных случаев были мутированными и 5 — немутированными. Все немутированные случаи (все из heteroHCDR3-подгруппы) имели сходство HCDR3 с ранее описанными последовательностями. Различия в общей выживаемости (OS), длительности периода до прогрессии заболевания (PFS) и начала лечения (TFS) для *IGHV3-21*-позитивных больных были статистически незначимы по сравнению с пациентами с ХЛЛ с экспрессией других *IGHV*-генов. Указанные параметры также сравнивали между больными ХЛЛ с экспрессией мутированных *IGHV3-21*- и других *IGHV*-генов. Отличительной чертой пациентов с экспрессией *IGHV3*-гена была высокая встречаемость солидных опухолей. Они развились в 4 *IGHV3-21*-позитивных случаях (36,4%) и в 10 случаях с экспрессией других *IGHV*-генов (5,6%, $p = 0{,}0002$). Кроме того, в небольшой группе больных (6) с экспрессией мутированного *IGHV3-21*-гена у 3 возникли солидные опухоли и 1 пациента — синдром Рихтера. У больных с экспрессией немутированного *IGHV3-21*-гена определяли худшие показатели OS и PFS по сравнению с пациентами с экспрессией других немутированных *IGHV*-генов.** ***Выводы:*** **представленные данные согласуются с мнением о самостоятельном негативном прогностическом значении для больных с ХЛЛ экспрессии *IGHV3-21*-гена вне зависимости от его мутационного статуса. *IGHV3-21*-экспрессия была ассоциирована с развитием вторичных солидных опухолей. Выявленный высокий уровень гомологии в heteroHDR3s-подгруппе может свидетельствовать о возможном антигенном влиянии в дополнение к антигенному влиянию в homHCDR3-подгруппе, что было установлено ранее.**

Ключевые слова: **хронический лимфолейкоз, гены вариабельных участков тяжелых цепей иммуноглобулинов, *IGHV3-21*, прогностический фактор.**