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Molecular analysis of HLA genes and the role of their polymorphism in chronic hepatitis B virus infection

PHD THESIS SUMMARY

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Content

Introduction

Chronic hepatitis B virus (HBV) infection is still a major health problem, and the most recent estimates in evaluating the goal of limiting hepatitis B virus infection have not yielded the expected results [1]. The need to investigate new genetic markers that would help to obtain a predictive model in therapeutic management, but also in prevention, is urgently needed. Recent epidemiological studies conducted on the Romanian population indicate a prevalence of HBsAg (hepatitis B surface antigen) positivity of 5.59%, and the seroprevalence of hepatitis viruses is higher compared to Central and Western Europe [2] with a similar percentage of 5.1% HBsAg positive in the category of pregnant women [3].

The research idea of the present work was represented by the thorough investigation of the molecular mechanisms by the involvement of HLA genes that control the immune response in chronic HBV infection.

The MHC (major histocompatibility complex) region has been described as a "genome within a genome" [4], so that the complexity of HLA genetic polymorphism by characterizing genes, not only at the peptide binding site, offered the opportunity of the present study to describe the links between the HLA genetic profile and the assessment of genetic risk in susceptibility and persistence in chronic HBV infection, but also the assessment of genes involved in protection against infection.

HLA immunogenetics represents a fascinating area of research, but also a challenge, since the continuous dynamics of the discovery of new alleles implies new perspectives of approach in the accurate identification of nucleotide sequences and associated diseases. According to the most recent report, in December 2024, 28409 HLA class I alleles and 12594 HLA class II alleles have been identified and named [5].

Next Generation Sequencing (NGS) of the HLA genomic region together with the information obtained will allow the development of a population genetic profile algorithm, even if HLA genotyping does not yet represent, like other biomarkers, which are used in genetic screening for a specific mutation predictive of a pathology.

The impact of HLA genetic diversity in viral infections has been addressed by researchers from all over the world, in different geographical areas, populations and ethnicities with specific characteristics for each region.

In this context, the present thesis addresses for the first time in Romania the study of HLA genetic polymorphism at a high-resolution level and the association with chronic HBV infection, it being well known that the diversity of the HLA genetic profile is related to the

allelic frequency, and certain genes such as HLA-C present reduced diversity in the nucleotide sequence. The main objective of the study was to characterize the HLA molecular genetic profile and the specific positions of amino acid residues in the study population, which was divided into two major categories: the group with chronic HBV hepatitis and the group of healthy individuals, an objective achieved by obtaining discoveries that could be the basis for future large-scale studies.

Another research approach of the study was the identification in the study population of specific HLA genotypes that present the heterozygous advantage, providing protection against viral infection, but also of homozygous genotypes involved in the persistence of HBV infection. The scientific work also analyzed the correlations between the status of viral replication and the control of the immune response and the presence of a certain HLA genetic polymorphism. The opportunity to sequence the entire HLA region in patients with chronic HBV infection and understanding the immunological response mediated by HLA genes could effectively contribute to the implementation of prevention strategies, as HBV infection has many clinical forms, from acute infection to hepatocellular carcinoma.

State of the art

Hepatitis B virus infection and all the complications that can develop, chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and even death, have led researchers and clinicians around the world to investigate and discover new therapeutic strategies. The latest WHO reports from 2022 estimated that around 254 million people are chronically infected with HBV, and over 1.1 million people have developed complications that led to death [6].

In Romania, the most recent results from the period 2020-2023 of the prevalence of infectious serological markers in hepatitis B estimated that the prevalence of the positive anti-HBc antibody marker was 26.3% in the age group 18-69 years. Higher values were reported in the North-East region compared to the Center region (29.6% versus 19.4%). The reported HBsAg positivity was 6.2% for the 18-69 age group, suggesting a 1.3% increase since the 2006-2008 study [7]. The influence of genotypes on the response to antiviral therapy is most effective when studies are conducted and focus on a single ethnicity, as both genotype and duration of HBV infection differ between indigenous and imported genotypes [8]. Chronically infected patients with genotype A are less likely to develop hepatocellular carcinoma than those with genotype D [9].

In the Romanian population, the frequency of genotype A was found in patients with

inactive HBV carriers(10), in the Transylvanian area the investigated patients had predominantly genotype D, and in addition to the 2008 study, the most recent study that analyzed the prevalence of genotypes in a cohort of patients who, in addition to HBV infection, also had HIV co-infection demonstrated that the presence of genotype A is associated with long-term survival [11]. The molecular epidemiology of HBV genotypes and the analysis of the diversity of subgenotypes of each ethnic group and the integration with the clinical characteristics of the patients will contribute to improving the deciphering of the complexity of the mechanism of action of the hepatitis B virus and the immune response, offering in the future new perspectives of approach that will lead to the outline of a complete and complex immunogenetic profile.

The genes of the major histocompatibility complex (MHC) have the greatest genetic diversity and are the key to the immune response which, by presenting antigenic peptides to T cells, will have the remarkable ability to differentiate the body's own cells from foreign ones, and the study of HLA biology has led to the description of molecular and genetic mechanisms in various autoimmune and infectious diseases, in drug hypersensitivity, but also in determining longevity [12]. In defining HLA polymorphism, the need to standardize the naming and classification of alleles was foreseen as early as 1968 when a committee was first established, called by the WHO "Nomenclature Committee for HLA System Factors" [13]. The information background of HLA genetic polymorphism due to current sequencing methodologies requires permanent rigor in the analysis of nucleotide sequences, and the continuous discovery of new HLA allelic variants brings permanent challenges to the scientific community and clinical practice. Progress in HLA immunogenetics research and accuracy in the identification of allelic variants are due to new sequencing technologies that have allowed the characterization of the entire HLA genomic region. HLA genotyping has multiple applications, having a crucial role in solid organ transplantation and hematopoietic stem cell transplantation, but also in the association with over 100 diseases and even in pharmacogenomic applications. The frequency of HLA alleles varies in different ethnic groups and has a significant impact on the susceptibility to adverse drug reactions, and many studies have confirmed the importance of HLA polymorphisms in pharmacogenetics. Recent American College of Rheumatology guidelines recommend that as a precautionary measure, patients who are to receive allopurinol for gout treatment should be genotyped for HLA-B*58:01, as carriers of this gene are at increased risk of developing severe cutaneous adverse reactions such as Stevens-Johnson syndrome [14].

In understanding the genetic basis and involvement of immunological responses in

susceptibility or protection against viral infections, numerous studies have evaluated HLA genes due to their very high polymorphism and the impact they have in generating immune responses. The complexity of HLA genetic polymorphism and its involvement in modulating the immune response has allowed researchers to investigate the interaction between the pathogenesis of viral infections and HLA genetic variants.

Personal contributions

Working hypothesis and general research objectives

The study of the association of HLA genetic polymorphisms with chronic HBV infection was approached in the context of the still high prevalence of the infection, despite the existence of an effective vaccine. A broader approach, which would include immunogenetic testing of individual peculiarities of the immune system, could provide additional information and the development of new strategies for addressing and monitoring HBV infection.

Main objectives of the research:

1. Analysis of HLA genetic profiles in chronic hepatitis B virus infection using the next-generation sequencing technique.
2. HLA genetic diversity and testing the heterozygous advantage theory in the context of chronic HBV infection.
3. Association of HLA genetic polymorphism with the effect of active viral replication of HBV DNA and the coexistence of hepatic steatosis.

Study group selection and description

Consecutive selection of patients with HBV infection from the Department of Gastroenterology and Hepatology, Fundeni Clinical Institute was carried out during 2022-2024, thus a total of 327 patients were eligible. The healthy control group consisted of 304 people (Table 4.1). The study complied with the Declaration of Helsinki, the rules and principles of good practice in research on human subjects and was approved by the Ethics Committee of the Fundeni Clinical Institute (28640/25.05.2022). The anonymized clinical data were harmonized with the information obtained in the laboratory, being entered into a database to which access was password protected to ensure data security and integrity.

Inclusion criteria: people over 18 years of age diagnosed with chronic HBV infection

based on virological, biochemical biomarkers and imaging investigations (abdominal ultrasound) and periodic monitoring every 6 months or 1 year.

Biological material

The biological material collected from the subjects included in the study consisted only of blood, so they were not exposed to risks. All participants included in the study, before signing the consent, were informed about the minimal potential risk that may occur during the collection of biological blood samples, a risk also provided for in the usual collection procedure during the periodic medical visits they perform. For the purpose of HLA genotyping, but also for the determination of HBV-DNA viremia, the primary sample collection consisted of peripheral blood collected on EDTA vacutainers.

Genomic DNA isolation from blood

The protocol for extracting total DNA from peripheral blood samples collected on vacutainer with EDTA was performed with the reagents from the kit. QIAmp DNA Blood Mini® kit (QIAGEN, Hilden, Germany) which is CE-IVD marked, in accordance with EU Directive 98/79/EC, according to the manufacturer's instructions. The reagents used were compatible with the QIAcube® equipment for performing the automated extraction which provides a clear advantage over manual extraction, avoiding cross-contamination, as the quality and purity of the DNA obtained is a key factor for next-generation sequencing.

Next-generation sequencing of genomic DNA for HLA allele genotyping

Amplification of the obtained genomic DNA in follow extraction for genotyping HLA alleles was performed USING MIA FORA™ NGS MFlex11 kit (Norcross, GA, USA) following instructions MANUFACTURER Immucor. The principle of the NGS method allows for the total coverage of HLA genes, including introns, 3'-UTR and 5'-UTR untranslated regions, but also upstream and downstream regions, consisting of the following major steps: long-range PCR, library construction, library preparation for sequencing. In a first step called long-range PCR in which rapid A DNA elongation takes place, combined with a small amount of polymerase proofreading for accuracy. Thus, the proofreading enzyme repairs DNA mismatches incorporated at the 3' end of the strand and allows elongation up to 30 kb. The next step consists of constructing bookstore established step that consists of the enzymatic fragmentation of the PCR products obtained in the long-range step. DNA fragmentation does not result in homogeneous fragments with blunt ends, they do not

contain unpaired bases at the 3 ' and 5 ' ends, therefore the ends of the DNA fragments are repaired and adenine is added to the 3 ' end , which allows the ligation of thymine at the 5' end of the adapter index. At the 3' end of the phosphorylated DNA fragment, deoxyadenosine 5'monophosphate to prevent concatemerization. The samples are purified and then follows ligating them with adapter index, after which the samples will be consolidated into a single tube.

Viral DNA isolation

Viral DNA extraction from samples collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant was performed with the commercial reagents Bosphore® Viral DNA Extraction Spin Kit (Anatolia Geneworks, Turkey, Istanbul). The kit is CE-IVD marked, in accordance with EU Directive 98/79/EC and was used according to the manufacturer's specifications.

Detection and quantification of HBV DNA by real-time PCR technique

Viral DNA obtained from extraction for detection and quantification of HBV DNA viral load was performed using the Bosphore HBV Quantification Kit and the Montania 4896 instrument (Anatolia Geneworks, Turkey, Istanbul) according to the manufacturer's instructions.

Analysis of virological markers

Collection and processing of biological samples for testing viral markers (Hbs Ag, anti-HBs, anti-HBc, Hbe Ag, anti-Hbe) was performed by collecting peripheral blood in vacutainer without anticoagulant with or without separator gel and centrifuging the blood samples for 30 minutes at 14000 rpm. The examination method was chemiluminescence using the Architect i2000 equipment (Abbott Laboratories, North Chicago, IL, USA) with compatible commercial reagents.

Biochemical marker analysis

Biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase, total bilirubin, direct bilirubin, alkaline phosphatase were determined by spectrophotometric method using Dimension® EXL™ 200 equipment (Siemens Healthcare GmbH, Germany, Forchheim).

Monitoring hepatic steatosis

Abdominal ultrasound examination along with the SteatoTest test (BioPredictive, Paris, France) contributed to the classification of stages of hepatic steatosis.

Statistical analysis of data

In this study, immunogenetic analysis was performed using MIDAS HLA (Molecular Immunogenetics Data Analysis System) version 1.10.0 comprising Bioconductor software version 3.18, as well as Microsoft Office Excel, using logistic regression models. The statistical interpretation for the calculation of variables of interest included: Odd ratio, 95% confidence intervals, p-value, Benjamini-Hochberg correction method**.**

Results

Analysis of variability in molecular profiles of HLA genes

In the evaluation of the association of HLA genetic polymorphisms and viral infection, a total of 247 patients with chronic HBV infection were included; the patient group consisted of 113 women aged between 23 and 81 years, and 134 men with a minimum age of 23 years and a maximum age of 83 years. The control group consisted of 304 healthy volunteers (118 women and 186 men) with or without hepatitis B vaccine.

The genetics of HLA-A allele variability led to the identification of 41 genetic variants, and by comparing the two study groups, a single allelic variant was found to have statistical significance, HLA-A*24:02:01 (p = 0.014) presenting a potential risk effect, with a confidence interval ranging from 1.11 - 2.53 and an OD with a value of 1.67 (Table 5.1). The interaction between HLA-A and the two studied categories led to the identification of three allelic variants with a tendency to be statistically significant, namely a variant with a possible protective effect against HBV infection, HLA-A*25:01:01 (p = 0.058; OD = 0.46; 95% CI = 0.20 - 1.05), and two other alleles with a potential risk role, HLA-A*30:02:01 (p = 0.085; OD = 3.73) and HLA-A*33:01:01 (p = 0.060; OD = 2.5) (Figure 5.1).

Table 5.1. Genetic polymorphism of HLA-A alleles in the patient group and the

control group

HLA-A	Patient group (2n = 494)		Control group (2n = 608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:01:01	67	13.56	87	14.31	0.722	0.94	0.67	1.32
*02:01:01	130	26.32	166	27.30	0.713	0.95	0.73	1.24
*02:05:01	5	1.01	2	0.33	0.156	3.10	0.60	16.04
*02:06:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*02:11:01	4	0.81	2	0.33	0.281	2.47	0.45	13.56
*02:17:02	1	0.20	3	0.49	0.424	0.41	0.04	3.94
*03:01:01	50	10.12	63	10.36	0.896	0.97	0.66	1.44
*03:01:71	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*03:02:01	1	0.20	5	0.82	0.164	0.24	0.03	2.10
*11:01:01	40	8.10	47	7.73	0.822	1.05	0.68	1.63
*23:01:01	9	1.82	21	3.45	0.098	0.52	0.24	1.14
***24:02:01**	**57**	**11.54**	**44**	**7.24**	**0.014**	**1.67**	**1.11**	**2.53**
*24:03:01	3	0.61	3	0.49	0.798	1.23	0.25	6.13
***25:01:01**	**8**	**1.62**	**21**	**3.45**	**0.058**	**0.46**	**0.20**	**1.05**
*26:01:01	29	5.87	30	4.93	0.492	1.20	0.71	2.03
*29:01:01	3	0.61	5	0.82	0.676	0.74	0.18	3.10
*29:02:01	4	0.81	7	1.15	0.571	0.70	0.20	2.41
*30:01:01	5	1.01	10	1.64	0.367	0.61	0.21	1.80
***30:02:01**	**6**	**1.21**	**2**	**0.33**	**0.085**	**3.73**	**0.75**	**18.54**
*31:01:02	9	1.82	10	1.64	0.822	1.11	0.45	2.75
*32:01:01	16	3.24	26	4.28	0.371	0.75	0.40	1.41
***33:01:01**	**12**	**2.43**	**6**	**0.99**	**0.060**	**2.50**	**0.93**	**6.70**
*33:03:01	4	0.81	7	1.15	0.571	0.70	0.20	2.41
*66:01:01	2	0.40	3	0.49	0.828	0.82	0.14	4.93
*68:01:01	8	1.62	7	1.15	0.505	1.41	0.51	3.92
*68:01:02	11	2.23	13	2.14	0.920	1.04	0.46	2.35
*68:02:01	2	0.40	3	0.49	0.828	0.82	0.14	4.93

*74:03:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80

The very high polymorphism of the HLA-B gene and the genetic information obtained by sequencing the 7 exons allowed the identification of 68 different allelic variants (Table 5.2). Among all the identified alleles, HLA-B*15:01:01 is statistically significant (p = 0.033; OD = 0.40) and can be classified as a protective genetic variant, an aspect also confirmed by the confidence interval (95% CI = 0.17 – 0.95). Also, from the HLA-B*15 group, but the variant *15:18:01 showed statistical significance (p = 0.013) as having a protective effect but this aspect was not confirmed by either the confidence interval or the odds ratio. On the other hand, another potential protective allelic variant was HLA-B*18:01:01 (p = 0.025; OD = 0.62; 95%CI = 0.41 – 0.94), a significance also supported by the confidence interval estimating the precision of OD. Moreover, 3 allelic variants with a potential protective effect were observed HLA-B*13:02:01 (p = 0.087; OD = 0.47), HLA-B*40:01:02 (p = 0.074; OD = 0.27), HLA-B*58:01:01 (p = 0.076; OD = 0.33) and a risk variant on HBV infection, namely HLA-B*14:02:01 (p = 0.083; OD = 2.0).

Table 5.2. Genetic polymorphism of HLA-B alleles in the patient group and the control group

HLA-B	Patient group (2n = 494)		Control group (2n = 608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*07:02:01	18	3.64	31	5.10	0.244	0.70	0.39	1.27
*07:05:01	2	0.40	2	0.33	0.835	1.23	0.17	8.78
*08:01:01	41	8.30	50	8.22	0.964	1.01	0.66	1.55
***13:02:01**	**7**	**1.42**	**18**	**2.96**	**0.087**	**0.47**	**0.20**	**1.14**
***14:02:01**	**16**	**3.24**	**10**	**1.64**	**0.083**	**2.00**	**0.90**	**4.45**
***15:01:01**	**7**	**1.42**	**21**	**3.45**	**0.033**	**0.40**	**0.17**	**0.95**
*15:08:01	2	0.40	1	0.16	0.446	2.47	0.22	27.29
*15:17:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80
*15:73:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
***18:01:01**	**37**	**7.49**	**70**	**11.51**	**0.025**	**0.62**	**0.41**	**0.94**
*18:03:01	4	0.81	2	0.33	0.281	2.47	0.45	13.56

*18:05:01	4	0.81	3	0.49	0.511	1.65	0.37	7.39
*27:02:01	10	2.02	10	1.64	0.639	1.24	0.51	0.99
*27:05:02	19	3.85	13	2.14	0.093	1.83	0.89	3.75
*35:01:01	31	6.28	38	6.25	0.986	1.00	0.62	1.64
*35:02:01	8	1.62	7	1.15	0.505	1.41	0.51	3.92
*35:03:01	21	4.25	36	5.92	0.213	0.71	0.41	1.22
*35:08:01	8	1.62	13	2.14	0.531	0.75	0.31	1.83
*37:01:01	7	1.42	7	1.15	0.695	1.23	0.43	3.54
*38:01:01	10	2.02	17	2.80	0.410	0.72	0.33	1.58
*39:01:01	13	2.63	12	1.97	0.466	1.34	0.61	2.97
*39:06:02	2	0.40	1	0.16	0.446	2.47	0.22	27.29
*39:24:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
***40:01:02**	**2**	**0.40**	**9**	**1.48**	**0.074**	**0.27**	**0.06**	**1.26**
*40:02:01	11	2.23	9	1.48	0.356	1.52	0.62	3.69
*40:06:01	9	1.82	5	0.82	0.141	2.24	0.75	6.72
*41:01:01	12	2.43	7	1.15	0.105	2.14	0.84	5.47
*41:02:01	2	0.40	2	0.33	0.835	1.23	0.17	8.78
*44:02:01	20	4.05	19	3.13	0.409	1.31	0.69	2.48
*44:03:01	18	3.64	18	2.96	0.526	1.24	0.64	2.41
*44:03:02	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*44:05:01	8	1.62	7	1.15	0.505	1.41	0.51	3.92
*44:27:01	5	1.01	9	1.48	0.490	0.68	0.23	2.04
*49:01:01	9	1.82	10	1.64	0.822	1.11	0.45	2.75
*50:01:01	4	0.81	4	0.66	0.768	1.23	0.31	4.95
*51:01:01	60	12.15	63	10.36	0.350	1.20	0.82	1.74
*51:07:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80
*51:65	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*52:01:01	19	3.85	15	2.47	0.188	1.58	0.80	3.15
*53:01:01	3	0.61	2	0.33	0.494	1.85	0.31	11.12
*55:01:01	7	1.42	4	0.66	0.207	2.17	0.63	7.46
*56:01:01	5	1.01	5	0.82	0.741	1.23	0.35	4.28
*57:01:01	13	2.63	15	2.47	0.863	1.07	0.50	2.27

***58:01:01**	**3**	**0.61**	**11**	**1.81**	**0.076**	**0.33**	**0.09**	**1.20**
*73:01:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74

The polymorphism of the HLA-C gene is lower than the polymorphism of the HLA-B gene (68 allelic variants), and was represented by a total number of 41 allelic variants (Table 5.3). However, according to the statistical significance (p value <0.05) for HLA-C no alleles were identified that had statistical power, but instead 3 allelic variants were found with a tendency to approach the statistical significance range: HLA-C*03:04:01 (p = 0.051; OD = 0.38; 95% CI = 0.14 – 1.04), HLA-C*15:02:01 (p = 0.058; OD = 1.60; 95% CI = 0.98 -2.60), HLA-C*07:01:02 (p = 0.075; OD = 2.80; 95% CI = 0.86-9.15).

Table 5.3. Genetic polymorphism of HLA-C alleles in the patient group and the control group

HLA-C	Patient group (2n = 494)		Control group (2n = 608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:02:01	24	4.86	27	4.44	0.743	1.10	0.63	1.93
*02:02:02	40	8.10	34	5.59	0.098	1.49	0.93	2.39
*03:02:02	2	0.40	7	1.15	0.171	0.35	0.07	1.69
*03:03:01	13	2.63	12	1.97	0.466	1.34	0.61	2.97
***03:04:01**	**5**	**1.01**	**16**	**2.63**	**0.051**	**0.38**	**0.14**	**1.04**
*04:01:01	75	15.18	98	16.12	0.671	0.93	0.67	1.29
*05:01:01	19	3.85	16	2.63	0.253	1.48	0.75	2.91
*06:02:01	32	6.48	49	8.06	0.317	0.79	0.50	1.25
*07:01:01	72	14.57	103	16.94	0.285	0.84	0.60	1.16
***07:01:02**	**9**	**1.82**	**4**	**0.66**	**0.075**	**2.80**	**0.86**	**9.15**
*07:02:01	26	5.26	36	5.92	0.637	0.88	0.53	1.48
*07:04:01	12	2.43	13	2.14	0.747	1.14	0.52	2.52
*07:06:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*07:18:01	3	0.61	3	0.49	0.798	1.23	0.25	6.13
*08:02:01	16	3.24	11	1.81	0.127	1.82	0.84	3.95

*12:02:02	17	3.44	14	2.30	0.256	1.51	0.74	3.10
*12:03:01	48	9.72	77	12.66	0.125	0.74	0.51	1.09
*14:02:01	12	2.43	17	2.80	0.705	0.87	0.41	1.83
***15:02:01**	**39**	**7.89**	**31**	**5.10**	**0.058**	**1.60**	**0.98**	**2.60**
*15:05:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*15:05:02	2	0.40	3	0.49	0.828	0.82	0.14	4.93
*15:06:01	2	0.40	1	0.16	0.446	2.47	0.22	27.29
*16:01:01	6	1.21	6	0.99	0.717	1.23	0.40	3.85
*16:02:01	2	0.40	4	0.66	0.570	0.61	0.11	3.37
*16:04:01	2	0.40	3	0.49	0.828	0.82	0.14	4.93
*17:01:01	3	0.61	6	0.99	0.486	0.61	0.15	2.46
*17:03:01	2	0.40	4	0.66	0.570	0.61	0.11	3.37

The allelic diversity of the HLA-DPA1 gene being quite low, 13 genetic variants, only one allele was identified with statistical power having a possible risk effect in hepatitis B virus infection, HLA-DPA1*02:01:04 (p = 0.031; OD = 3.0; 95% CI = 1.05-8.58) (Table 5.4). Another observation refers to the tendency to be meaningful statistics the HLA-DPA1*02:07:01 allele as a risk allele (p = 0.075, OD =2.80).

Table 5. 4. Genetic polymorphism of HLA-DPA1 alleles in the patient group and the control group

HLA-DPA1	Patient group (2n=494)		Control group (2n=608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:03:01	374	75.71	486	79.93	0.092	0.78	0.59	1.04
*01:04:01	1	0.20	5	0.82	0.164	0.24	0.03	2.10
*02:01:01	56	11.34	73	12.01	0.731	0.94	0.65	1.36
*02:01:02	24	4.86	24	3.95	0.461	1.24	0.70	2.22
***02:01:04**	**12**	**2.43**	**5**	**0.82**	**0.031**	**3.00**	**1.05**	**8.58**
*02:02:02	12	2.43	9	1.48	0.252	1.66	0.69	3.97
***02:07:01**	**9**	**1.82**	**4**	**0.66**	**0.075**	**2.80**	**0.86**	**9.15**

In the case of the HLA-DPB1 gene, 34 allelic variants were observed (Table 5.5), and from a statistical point of view, according to the p-value less than 0.05, three alleles were highlighted as protective alleles against HBV infection: HLA-DPB1*09:01:01, HLA-DPB1*11:01:01, HLA-DPB1*23:01:01.

Table 5.5. Genetic polymorphism of HLA-DPB1 alleles in the patient group and the control group

HLA-DPB1	Patient group (2n=494)		Control group (2n=608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:01:01	25	5.06	27	4.44	0.629	1.15	0.66	2.00
*02:01:02	94	19.03	109	17.93	0.639	1.08	0.79	1.46
*03:01:01	30	6.07	27	4.44	0.224	1.39	0.82	2.37
***04:01:01**	**154**	**31.17**	**220**	**36.18**	**0.081**	**0.80**	**0.62**	**1.03**
*04:02:01	81	16.40	99	16.28	0.959	1.01	0.73	1.39
*05:01:01	9	1.82	8	1.32	0.498	1.39	0.53	3.63
*06:01:01	8	1.62	6	0.99	0.351	1.65	0.57	4.79
***09:01:01**	**2**	**0.40**	**10**	**1.64**	**0.049**	**0.24**	**0.05**	**1.11**
*10:01:01	16	3.24	13	2.14	0.256	1.53	0.73	3.22
*104:01:01	8	1.62	7	1.15	0.505	1.41	0.51	3.92
*13:01:01	21	4.25	18	2.96	0.249	1.46	0.77	2.76
*138:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80
*14:01:01	16	3.24	15	2.47	0.441	1.32	0.65	2.70
*15:01:01	5	1.01	5	0.82	0.741	1.23	0.35	4.28
*16:01:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80
*17:01:01	5	1.01	14	2.30	0.102	0.43	0.16	1.21
*19:01:01	3	0.61	1	0.16	0.224	3.71	0.38	35.77
***23:01:01**	**4**	**0.81**	**15**	**2.47**	**0.036**	**0.32**	**0.11**	**0.98**

Genotyping information obtained by next-generation sequencing allowed a detailed

evaluation of the 23 allelic variants belonging to the HLA-DQA1 gene (Table 5.6). HLA-DQA1*01:02:01 was associated as a protective gene (p<0.001; OR = 0.42), in contrast HLA-DQA1*01:02:02 (p = 0.002; OD = 1.71) suggests a potential risk association in case of chronic HBV infection. HLA-DQA1*01:03:01 confers potential protection (p = 0.002; OD = 0.44), on the other hand the allelic variant HLA-DQA1*01:01:02 (p = 0.042, OD = 2.5) correlated with susceptibility to HBV infection.

Table 5.6. Genetic polymorphism of HLA-DQA1 alleles in the patient group and the control group

HLA-DQA1	Patient group (2n = 494)		Control batch (2n = 608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:01:01	46	9.31	43	7.07	0.175	1.35	0.87	2.08
***01:01:02**	**14**	**2.83**	**7**	**1.15**	**0.042**	**2.50**	**1.00**	**6.25**
***01:02:01**	**23**	**4.66**	**63**	**10.36**	**0.000**	**0.42**	**0.26**	**0.69**
***01:02:02**	**84**	**17.00**	**65**	**10.69**	**0.002**	**1.71**	**1.21**	**2.42**
***01:03:01**	**20**	**4.05**	**53**	**8.72**	**0.002**	**0.44**	**0.26**	**0.75**
*01:04:01	18	3.64	23	3.78	0.903	0.96	0.51	1.80
*01:04:02	11	2.23	7	1.15	0.161	1.96	0.75	5.08
*01:05:01	7	1.42	12	1.97	0.480	0.71	0.28	1.83
*02:01:01	36	7.29	54	8.88	0.337	0.81	0.52	1.25
*03:01:01	24	4.86	38	6.25	0.319	0.77	0.45	1.30
*03:03:01	13	2.63	10	1.64	0.254	1.62	0.70	3.72
*04:01:01	5	1.01	8	1.32	0.642	0.77	0.25	2.36
*05:01:01	72	14.57	68	11.18	0.093	1.35	0.95	1.93
*05:03:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
*05:05:01	116	23.48	146	24.01	0.837	0.97	0.73	1.28
*06:01:01	1	0.20	2	0.33	0.689	0.61	0.06	6.80

In the assessment of the association of genetic polymorphisms, we identified, at high

resolution, 22 allelic variants of the DQB1 gene (Table 5.7). The significant associations taking into account the p value was represented by HLA-DQB1*05:02:01 (p = 0.003), being a possible risk allele. At the limit of statistical significance, the HLA-DQB1*05:01:01 variant (p = 0.069) was highlighted with possible involvement in the risk of HBV infection (OD = 1.41; 95% CI = 0.97 - 2.04). Two allelic variants were noted as having a highly statistically significant association with the protective effect: HLA-DQB1*06:02:01 (p < 0.001; OD = 0.21; 95% CI = 0.09 - 0.47) and HLA-DQB1*06:02:01 (p < 0.001; OD = 0.21; 95% CI = 0.09 - 0.47).

Table 5.7. Genetic polymorphism of HLA-DQB1 alleles in the patient group and the control group

HLA-DQB1	Patient group (2n = 494)		Control batch (2n = 608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*02:01:01	72	14.57	68	11.18	0.093	1.35	0.95	1.93
*02:02:01	27	5.47	41	6.74	0.381	0.80	0.48	1.32
*02:10	1	0.20	0	0.00	0.267	-	-	-
*03:01:01	127	25.71	153	25.16	0.837	1.03	0.78	1.35
*03:02:01	25	5.06	40	6.58	0.287	0.76	0.45	1.27
*03:03:02	9	1.82	17	2.80	0.289	0.65	0.29	1.46
*03:04:01	3	0.61	3	0.49	0.798	1.23	0.25	6.13
*04:02:01	5	1.01	11	1.81	0.271	0.55	0.19	1.61
***05:01:01**	**67**	**13.56**	**61**	**10.03**	**0.069**	**1.41**	**0.97**	**2.04**
***05:02:01**	**87**	**17.61**	**69**	**11.35**	**0.003**	**1.67**	**1.19**	**2.35**
*05:03:01	32	6.48	33	5.43	0.462	1.21	0.73	1.99
*06:01:01	11	2.23	10	1.64	0.482	1.36	0.57	3.23
***06:02:01**	**7**	**1.42**	**39**	**6.41**	**<0.001**	**0.21**	**0.09**	**0.47**
***06:03:01**	**8**	**1.62**	**45**	**7.40**	**<0.001**	**0.21**	**0.09**	**0.44**
*06:04:01	6	1.21	12	1.97	0.323	0.61	0.23	1.64
*06:09:01	1	0.20	3	0.49	0.424	0.41	0.04	3.94

The HLA-DRB1 gene presented great diversity, with 42 allelic variants identified (Table 5.8) and four specific alleles with a risk effect on HBV infection were identified, HLA-DRB1*01:02:01, HLA-DRB1*04:07:01, HLA-DRB1*16:01:01 HLA-DRB1*16:01:01 and 3 genetic variants with a protective role HLA-DRB1*04:02:01, HLA-DRB1*13:01:01, HLA-DRB1* 15:01:01. Three other alleles may suggest a potential protective role, HLA-DRB1*13:01:01 ($p < 0.001$; OD = 0.23; 95% CI = 0.11 - 0.5) and HLA-DRB1*15:01:01 ($p = 0.001$; OD = 0.38; 95% CI = 0.21 – 0.68) and DRB1*01:02:01 ($p = 0.042$; OD = 2.5; 95% CI = 1.0 – 6.25).

Table 5.8. Genetic polymorphism of HLA-DRB1 alleles in the patient group and the control group

HLA-DRB1	Patient group (2n=494)		Control group (2n=608)		*p*	OR	95% CI	
	Total	%	Total	%			Lower	higher
*01:01:01	47	9.51	43	7.07	0.141	1.38	0.90	2.13
***01:02:01**	**14**	**2.83**	**7**	**1.15**	**0.042**	**2.50**	**1.00**	**6.25**
*03:01:01	69	13.97	67	11.02	0.139	1.31	0.92	1.88
*04:01:01	6	1.21	10	1.64	0.553	0.74	0.27	2.04
***04:02:01**	**3**	**0.61**	**11**	**1.81**	**0.076**	**0.33**	**0.09**	**1.20**
*04:03:01	7	1.42	7	1.15	0.695	1.23	0.43	3.54
*04:04:01	10	2.02	8	1.32	0.356	1.55	0.61	3.96
*04:05:01	5	1.01	5	0.82	0.741	1.23	0.35	4.28
***04:07:01**	**5**	**1.01**	**1**	**0.16**	**0.057**	**6.21**	**0.72**	**53.30**
*04:08:01	2	0.40	4	0.66	0.570	0.61	0.11	3.37
*07:01:01	36	7.29	54	8.88	0.337	0.81	0.52	1.25
*08:01:01	5	1.01	9	1.48	0.490	0.68	0.23	2.04
*10:01:01	7	1.42	12	1.97	0.480	0.71	0.28	1.83
*11:01:01	44	8.91	49	8.06	0.615	1.12	0.73	1.71
*11:03:01	2	0.40	7	1.15	0.171	0.35	0.07	1.69

*11:04:01	51	10.32	68	11.18	0.647	0.91	0.62	1.34
*12:01:01	14	2.83	14	2.30	0.577	1.24	0.58	2.62
*12:02:01	1	0.20	1	0.16	0.883	1.23	0.08	19.74
***13:01:01**	**8**	**1.62**	**40**	**6.58**	**<0.001**	**0.23**	**0.11**	**0.50**
*13:02:01	7	1.42	17	2.80	0.119	0.50	0.21	1.21
*13:03:01	6	1.21	9	1.48	0.705	0.82	0.29	2.31
*14:01:01	5	1.01	3	0.49	0.313	2.06	0.49	8.67
*14:04:01	11	2.23	7	1.15	0.161	1.96	0.75	5.08
*14:54:01	13	2.63	21	3.45	0.432	0.76	0.37	1.52
***15:01:01**	**16**	**3.24**	**49**	**8.06**	**0.001**	**0.38**	**0.21**	**0.68**
*15:02:01	14	2.83	13	2.14	0.457	1.33	0.62	2.87
***16:01:01**	**73**	**14.78**	**57**	**9.38**	**0.006**	**1.68**	**1.16**	**2.42**
*16:02:01	7	1.42	3	0.49	0.108	2.90	0.75	11.27

The HLA-DRB3/4/5 genes, although the variability is much lower and their presence is dependent on HLA-DRB1, in the investigation of the immunological role, statistically significant correlations were found. Thus, 9 allelic variants for HLA-DRB3 were determined, of which 9 allelic variants with a significant impact in the association with a possible risk effect in HBV infection were HLA-DRB3*02:02:01 ($p = 0.010$; OD = 1.59; 95% CI = 1.12 – 2.27), and the variant HLA-DRB3*01:01:02 ($p = 0.023$, OD = 0.64; 95% CI = 1.12 – 2.27). At the opposite pole was HLA-DRB4 for which although 5 different allelic variants were identified, none had statistical significance. In the case of the HLA-DRB5 gene, a total of 5 allelic variants were determined, and a positive correlation was represented by HLA-DRB5*01:01:01 ($p < 0.001$; OD = 0.28; 95% CI = 0.15 – 0.52), the presence of this allele being higher in the group in which subjects without HBV infection were included, and the genetic variant HLA-DRB5*02:02:01 ($p = 0.004$; OD = 2.22; 95% CI =1.29 – 3.82) was highlighted as having statistical relevance in the risk of HBV infection.

Characterization of rare alleles found in the study cohort using next-generation sequencing

The accuracy of genotyping results through next-generation sequencing allowed the characterization of HLA gene diversity and the depth of polymorphisms at the exon and intron levels for understanding molecular mechanisms in population genetics, as well as the

association with various viral infections. Most studies have analyzed the associations between common and well-documented alleles, both in transplantation and in the association of different pathologies. Another approach of the present study focused on the correct identification of rare alleles, which unlike being classified as a common allele, that is, that allele has been identified at least 3 times in 3 different populations, and the term "well-documented" refers to the reporting of at least 5 occurrences in the same population, but at the level of unrelated individuals, the characterization of rare alleles in the Romanian population allows the understanding of immunological mechanisms. Thus, the following rare alleles were identified: HLA-A*03:20, HLA-A*02:436, HLA-A*11:327, HLA-A*26:15, HLA-B*35:370, HLA-B*35:109, HLA-C*04:29, HLA-DPB1*519:01, HLA-DPB1*650:01

Genetic diversity and testing the hypothesis of the advantage of heterozygosity of HLA genes

To assess the distribution of HLA diversity and test the effect of heterozygous advantage, 327 patients with chronic HBV infection (patient group) and 304 healthy subjects (control group) were analyzed. Homozygosity was defined as the presence of two identical allelic variants for an HLA gene, and individuals possessing two different allelic variants of the same gene were classified as heterozygous.

In assessing the impact of HLA-A zygosity, allelic variants with a frequency greater than 2% were considered. The heterozygous genotype for HLA-A*25:01:01 (p = 0.011; OD = 0.37; 95% CI = 0.17–0.83) provided a significant advantage in terms of protective role. Homozygosity for HLA-A*01:01:01 was negatively associated with the probability of protection (p = 0.033; OD = 2.35; 95% CI = 1.06–5.2).

A potential positive correlation in terms of protection against HBV infection is suggested by the heterozygosity of allelic variants that approached the limit of statistical significance HLA-B*07:02:01 (p = 0.058; OD = 0.57; 95% CI = 0.32-1.03), HLA-B*58:01:01 (p = 0.057; OD = 0.34; 95% CI = 0.11-1.09) and HLA-B*40:01:02 (p = 0.069; OD = 0.32; 95% CI = 0.09-1.17). In the analysis of the effect of homozygosity, a single allelic variant was statistically significant HLA-B*18:01:01 (p = 0.001; OD = 0.15; 95% CI = 0.05-0.47) being classified as a potential protective allele. The data examined at the level of HLA-DPB1 genotyping suggest that heterozygosity for HLA-DPB1*09:01:01 provides an important implication in protection against HBV (p = 0.036; OD = 0.27; 95% CI = 0.07-1.0), as well as for HLA-DPB1*23:01:01 (p = 0.056; OD = 0.42; 95% CI = 0.17 - 1.05),

these two allelic variants were not present in homozygous individuals. Regarding the heterozygous effect of the allelic variant HLA-DQB1*06:02:01 and HLA-DQB1*06:03:01, these variants were positively correlated with protection, this significance being emphasized by the observation that these two gene variants do not present homozygous status in the studied population.

Association of HLA genotyping with virological response in chronic HBV hepatitis

In identifying HLA genetic variations involved in viral replication, we analyzed 204 chronic HBV patients who were divided into two large categories according to viremia values, thus 28 patients were included in the group with HBV DNA < 2000 IU/mL and 176 patients in the group with HBV DNA ≥ 2000 IU/mL and without antiviral treatment. It was found that two HLA allelic variants were negatively correlated with low levels of HBV DNA, such as HLA-A*02:01:01 ($p = 0.060$), this allele was present in 40.74% of patients with low viremia, and the HLA-B*18:01:01 ($p = 0.0417$) allelic variant was identified in 29.63% of patients. Regarding HLA-DQB1*05:02:01, it was observed that 38.98% of patients presented with increased levels of HBV DNA ($p = 0.014$). HLA-DRB1*16:01:01 and HLA-DRB5*02:02:01 were found to be positively correlated with the presence of an increased level of HBV DNA in the patients studied.

Conclusions and personal contributions

The research in this paper focused on the molecular characterization of the HLA genetic profile of patients with chronic hepatitis B virus infection using next-generation sequencing, providing comprehensive information about the highly polymorphic nature of the HLA region. Thus, the challenge of precisely and accurately identifying HLA genes, even rare allelic variants and those whose polymorphism is outside exon 2 and 3, brings value in understanding the biological variation of the HLA system and the immune response, being the first research on the Romanian population that addressed high-resolution HLA genotyping in the context of chronic HBV infection. The investigations carried out within the framework of this thesis were structured and carried out in essential stages, each of which has a well-defined role in achieving the objectives.

The analysis of the involvement of specific HLA genotypes in chronic HBV infection

revealed statistically significant results, thus alleles strongly associated with the risk of chronic HBV infection were identified. Class I genes present a lower polymorphism unlike genes belonging to class II, thus the only allelic variant involved in chronic HBV infection was HLA-A*24:02:01.

Allelic variants of the HLA-DQ gene were shown to be positively associated with susceptibility to chronic HBV infection, and the most statistically significant alleles were HLA-DQA1*01:01:02, HLA-DQA1*01:02:02 and HLA-DQB1*05:02:01. A very important observation highlights the utility of NGS technology, which allowed the complete mapping of the alignment of all nucleotide sequences and was able to differentiate changes at the protein sequence level. The two variants belonging to the DQA1*01 allelic group show differences with an impact on protein function (glutamic acid for DQA1*01:01:02 and glutamine for DQA1*01:02:02).

Polymorphisms of the HLA-DR gene have a molecular signature and influence the immune response, and the genotypes HLA-DRB1*16:01:01, HLA-DRB3*02:02:01 and HLA-DRB5*02:02:01 have been shown to be involved in the risk of chronic HBV infection. The presence of the HLA-DPA1*02:01:04 genetic variant was significantly correlated with the risk of chronic HBV infection. Comparison of HLA genes in patients with chronic HBV infection and the healthy group without the presence of HBV infection highlights the protective role of allelic variants belonging to HLA class I genes, namely HLA-B*15:01:01, HLA-B*18:01:01, HLA-C*03:04:01.

An important genetic association in the immunological control of HBV infection was represented by the polymorphism of the HLA-DR gene, more precisely the alleles HLA-DRB1*13:01:01, HLA-DRB1*15:01:01, HLA-DRB3*01:01:02 and HLA-DRB5*01:01:01 were negatively correlated, their role being a protective one regarding HBV infection.

Statistically significant importance on the protective role was represented by four allelic variants of the HLA-DQ gene, namely HLA-DQA1*01:02:01, HLA-DQA1*01:03:01, HLA-DQB1*06:02:01, HLA-DQB1*06:03:01, but also by two alleles of the HLA-DP gene, namely HLA-DPB1*09:01:01 and HLA-DPB1*23:01:01. The use of NGS technology highlighted the importance of accurately identifying HLA alleles and synonymous mutations, so if the HLA-DQA1*01:02:01 allele was classified as a risk allele in chronic HBV infection, on the other hand the HLA-DQA1*01:02:02 allele has a protective role against HBV, although the difference between the two alleles does not produce changes at the protein sequence level, the SNP between the two alleles leading to the encoding of the same amino acid isoleucine.

The evaluation of the results of the present study brings a new perspective on the impact of HLA gene zygosity. Heterozygous genotypes for HLA-A*25:01:01, HLA-B*07:02:01, HLA-B*58:01:01, HLA-B*40:01:02, HLA-DPB1*09:01:01, HLA-DPB1*23:01:01, HLA-DQA1*01:02:01, HLA-DQB1*06:02:01, HLA-DQB1*06:03:01, HLA-DRB5*01:01:01 confirmed the theory of heterozygosity advantage, presenting important significance in the role of protection against HBV infection. Homozygous genotypes for HLA-A*24:02:01, HLA-DQA1*01:02:02, HLA-DQB1*05:01:01, HLA-DQB1*05:02:01 have significant relevance in the risk of chronic HBV infection.

In the context of the association between HLA genes and viral replication, an important relevance regarding increased levels of viral load, four allelic variants HLA-DQA1*01:02:02, HLA-DQB1*05:02:01, HLA-DRB1*16:01:01, HLA-DRB5*02:02:01 were significantly associated. Conversely, low levels of HBV DNA were reported to be correlated with HLA-A*02:01:01 and HLA-B*18:01:01.

Stratification of patients based on the presence/absence of hepatic steatosis contributed to the phenotypic variability of the disease, so that the HLA-B*08:01:01, HLA-C*07:01:01 and HLA-DRB3*01:01:02 alleles were identified as genetic risk factors for the presence of steatosis in patients with chronic HBV infection, and HLA-A*03:01:01, HLA-A*26:01:01 were positively associated with the absence of steatosis.

The results obtained in this paper emphasize the importance of the complexity of the mechanism by which HLA genes influence the immune response in chronic hepatitis B virus infection, and the contouring of a complete immunological profile will determine the HLA genetic signature taking into account the particular characteristics of each person. Incorporating the results on HLA genetic polymorphism together with the current biomarkers used in the management of HBV infection will create new opportunities in the development, optimization of therapeutic targets and an integrated multidisciplinary management.

Selective bibliography

1. Razavi-Shearer DM, Gamkrelidze I, Pan CQ, Jia J, Berg T, Gray RT, et al. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modeling study. Lancet Gastroenterol Hepatol. 2023 Oct; 8:879–907.
2. Voiculescu M, Iliescu L, Ionescu C, Micu L, Ismail G, Zilisteanu D, et al. A cross-sectional epidemiological study of HBV, HCV, HDV and HEV prevalence in the SubCarpathian and South-Eastern regions of Romania. Journal of Gastrointestinal and

Liver Diseases. 2010; 19:43–8.

3. Popovici O, Radu R, Romaniuc A, Azoicăi D. A seroprevalence study for hepatitis B virus markers of infection in pregnant women in Romania: Results and opportunities for prevention. Journal of Gastrointestinal and Liver Diseases. 2018 Jun; 27:133–7.
4. Gourraud PA, Khankhanian P, Cereb N, Yang SY, Feolo M, Maiers M, et al. HLA diversity in the 1000 genomes dataset. PLoS One. 2014 Jul;9.
5. https://hla.alleles.org/nomenclature/stats.html [Internet]. HLA Allele Numbers.
6. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b [Internet]. Hepatitis B.
7. https://insp.gov.ro/download/analiza-rezultatelor-studiului-sero-epidemiologic-de-prevalenta-pentru-markeri-de-infectie-cu-virus-hepatitic-b-si-virus-hepatitic-c_2022-2023/ [Internet]. National Institute of Public Health.
8. Kobayashi M, Suzuki F, Akuta N, Suzuki Y, Arase Y, Ikeda K, et al. Response to long-term lamivudine treatment in patients infected with hepatitis B virus genotypes A, B, and C. J Med Virol. 2006 Oct; 78:1276–83.
9. Asim M, Sarma MP, Kar P. Etiological and molecular profile of hepatocellular cancer from India. Int J Cancer. 2013 Jul; 133:437–45.
10. Constantinescu I, Nedelcu F, Toader MA, Daniela V. Clinical and therapeutic importance of HBV genotyping in Romania. J Med Life. 2008; 1:165–73.
11. Sultana C, Casian M, Oprea C, Ianache I, Grancea C, Chiriac D, et al. Hepatitis B Virus Genotypes and Antiviral Resistance Mutations in Romanian HIV-HBV Co-Infected Patients. Medicina (Lithuania). 2022 Apr;58.
12. Dendrou CA, Petersen J, Rossjohn J, Fugger L. HLA variation and disease. Vol. 18, Nature Reviews Immunology. Nature Publishing Group; 2018. p. 325–39.
13. https://hla.alleles.org/nomenclature/nomenc_reports.html [Internet]. Nomenclature for Factors of the HLA System.
14. FitzGerald JD, Dalbeth N, Mikuls T, Brignardello-Petersen R, Guyatt G, Abeles AM, et al. 2020 American College of Rheumatology Guideline for the Management of Gout. Arthritis and Rheumatology. 2020 Jun; 72:879–95.

List of scientific papers published

Tălăngescu A, Calenic B, Mihăilescu DF, Tizu M, Marunțelu I, Constantinescu AE, Constantinescu I, *Molecular Analysis of HLA Genes in Romanian Patients with Chronic Hepatitis B Virus Infection*, Current Issues in Molecular Biology, 2024, 46(2), 1064-1077, impact factor – 3,1/2024
https://doi.org/10.3390/cimb46020067

Tălăngescu A, Tizu M, Calenic B, Mihăilescu DF, Constantinescu AE, Constantinescu I, *HLA Genetic Diversity and Chronic Hepatitis B Virus Infection: Effect of Heterozygosity Advantage*, Medical Sciences, 2024, 12(3):44
https://doi.org/10.3390/medsci12030044

Tălăngescu A, Bratei AA, Tizu M, Calenic B, Constantinescu AE, Constantinescu I, *High-resolution genotyping of HLA alleles and association with genetic background of virological response in chronic hepatitis B*, Clinical Laboratory, 250119-07/2025 accepted for publication, impact factor – 0,7/2025