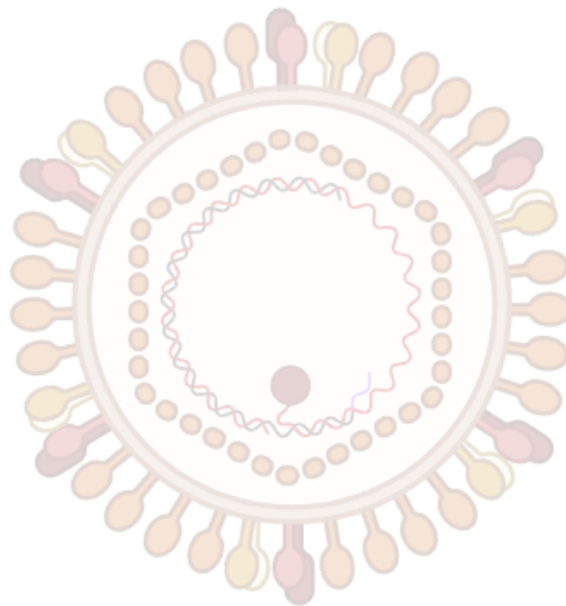


Summary of professional accomplishments

Dr Magda Rybicka-Misiejko



**Laboratory of Photobiology and Molecular Diagnostics
Intercollegiate Faculty of Biotechnology University of Gdańsk
and Medical University of Gdańsk**

Gdańsk, 2025

1. Name.

Magda Rybicka-Misiejko, <https://orcid.org/0000-0003-3768-3514>

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, and title of the Ph.D. dissertation.

- **2015:** Ph.D. in Biological Sciences in the field of Biochemistry Intercollegiate Faculty of Biotechnology UG and MUG. Title of the Ph.D. dissertation: „The influence of hepatitis B virus (HBV) polymorphism on the response to antiviral treatment in chronically infected patients”.
- **2009:** Master's degree in Biotechnology, Intercollegiate Faculty of Biotechnology UG and MUG.
- **2007:** Bachelor's degree in Medical Biotechnology, The Nicolaus Copernicus University in Torun (NCU), Collegium Medicum in Bydgoszcz.

3. Information on employment in research institutes or faculties/departments or school of arts.

- **2021 – present** – Assistant Professor in the Department of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG and MUG
- **2018-2021** – Research and Technical Staff Member in the Specialist Laboratories Team, Intercollegiate Faculty of Biotechnology UG and MUG
- **2015-2017** – Researcher/Post-doc in the Department of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG and MUG
- **2014-2015** – Researcher/Assistant in the Department of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG and MUG

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

For the purposes of the habilitation procedure, I would like to highlight a series of eight thematically related publications as my main scientific achievement, including six experimental papers. These publications examine **the impact of genetic and molecular factors on chronic hepatitis B virus (HBV) infection and its diagnosis**. I served as the lead or corresponding author in most of the publications (with the exception of one), which highlights my significant contribution to conducting the research and developing the results. None of the publications in this series have previously been used in proceedings for the award of a doctoral or habilitation degree. My other scientific

achievements from the period following the award of my doctorate are discussed in **sections 4.4 and 4.5 of this document.**

4.1 Title of the scientific achievement

Identification of viral factors and genetic host conditions in relation to the progression and treatment of chronic hepatitis B.

4.2 List of Scientific Publications Forming the Basis of the Scientific Achievement

The Impact Factor (IF) and the scoring by the Ministry of Education and Science (MEiN) are consistent with the year of publication of the works. However, the ministerial scoring system has evolved over time, affecting the evaluation of works from different periods.

PUBLICATION 1

Current molecular methods for the detection of hepatitis B virus quasispecies.

Rybicka M*, Stalke P, Bielawski KP.

Reviews in Medical Virology 2016 Sep;26(5):369-81. doi: [10.1002/rmv.1897](https://doi.org/10.1002/rmv.1897)

*corresponding author

IF₂₀₁₆=5,439; MEiN₂₀₁₆=45

Review article

My contribution to the publication included:

- Formulating the concept and outline of the article
- Conducting the literature review and selecting relevant sources
- Preparing the initial draft of the manuscript, including tables and figures
- Revising the manuscript in response to comments from journal reviewers
- Preparing responses to reviewers and corresponding with the editor
- Serving as the corresponding author

PUBLICATION 2

Differences in sequences between HBV-relaxed circular DNA and covalently closed circular DNA.

Rybicka M, Woziwodzka A, Romanowski T, Stalke P, Dręczewski M, Bielawski KP

Emerging Microbes and Infection 2017 Jun 21;6(6):e55. doi: [10.1038/emi.2017.41](https://doi.org/10.1038/emi.2017.41)

IF₂₀₁₇=6,032; MEiN₂₀₁₇=30

Original article

My contribution to the publication included:

- Participation in conceptualization and hypothesis development
- Involvement in developing and optimizing the research methodology

- Participation in all conducted experiments, from the isolation of HBV genetic material to the detection of HBV drug-resistant variants
- Contribution to the analysis and interpretation of the obtained results
- Conducting all statistical analyses
- Preparation of the initial draft of the manuscript, including tables and figures
- Taking a leading role in preparing responses to reviewers' comments and implementing revisions to the final accepted version of the article
- Securing funding for part of the research conducted

PUBLICATION 3

Host genetic background affects the course of infection and treatment response in patients with chronic hepatitis B.

Rybicka M*, Woziwodzka A*, Romanowski T, Sznarkowska A, Stalke P, Dręczewski M, Bielawski KP.

Journal of Clinical Virology 2019 Nov;120:1-5. doi: [0.1016/j.jcv.2019.09.002](https://doi.org/10.1016/j.jcv.2019.09.002)

* equal contribution of authors

IF₂₀₁₉=2,777; MEiN₂₀₁₉=100

Original article

My contribution to the publication included:

- Participation in formulating the research hypothesis and study concept
- Involvement in developing and optimizing the research methodology
- Participation in planning and conducting experiments
- Involvement in collecting research material
- Creation of a clinical and pathological database of patients
- Contribution to the interpretation and visualization of results
- Conducting statistical analyses of the obtained results
- Leading role in preparing the initial draft of the manuscript
- Active participation in responding to reviewers' comments and implementing required revisions to the final version

PUBLICATION 4

TNF- α polymorphisms affect persistence and progression of HBV infection.

Woziwodzka A, **Rybicka M**, Sznarkowska A, Romanowski T, Dręczewski M, Stalke P, Bielawski KP.

Molecular Genetics & Genomic Medicine 2019 Oct;7(10):e00935. doi: [10.1002/mgg3.935](https://doi.org/10.1002/mgg3.935)

IF₂₀₁₉=1,995; MEiN₂₀₁₉=70

Original article

My contribution to the publication included:

- Participation in formulating the research hypothesis and study concept
- Involvement in developing and optimizing the research methodology
- Participation in conducting experiments (i.e., DNA isolation, patient genotyping)

- Contribution to the analysis and interpretation of results
- Participation in writing the initial draft of the manuscript and revising the manuscript during the review process

PUBLICATION 5

Genetic variation in IL-10 influences the progression of hepatitis B infection.

Rybicka M*, Woziwodzka A, Sznarkowska A, Romanowski T, Stalke P, Dręczewski M, Verrier ER, Baumert TF, Bielawski KP*.

International Journal of Infectious Diseases 2020 Jul;96:260-265. doi: [10.1016/j.ijid.2020.04.079](https://doi.org/10.1016/j.ijid.2020.04.079)

*corresponding authors

IF=3,623; MEiN=100

Original article

My contribution to the publication included:

- Conceptualization and hypothesis development
- Development and optimization of the research methodology
- Participation in planning and conducting experiments
- Involvement in collecting research material
- Creation of a clinical and pathological database of patients
- Interpretation and visualization of results
- Conducting statistical analyses of the obtained results
- Preparation of the initial draft of the manuscript, including tables and figures
- Revising the manuscript in response to journal reviewers' comments
- Preparing responses to reviewers and corresponding with the editor
- Serving as the corresponding author

PUBLICATION 6

Recent Advances in Understanding, Diagnosing, and Treating Hepatitis B Virus Infection.

Rybicka M*, Bielawski KP.

Microorganisms 2020 Sep 15;8(9):1416. doi: [10.3390/microorganisms8091416](https://doi.org/10.3390/microorganisms8091416)

*corresponding author

IF=4,128; MEiN=40

Review article

My contribution to the publication included:

- Formulation of the concept and outline of the article
- Conducting the literature review and selecting relevant sources
- Preparation of the initial draft of the manuscript, including tables and figures
- Revising the manuscript in response to journal reviewers' comments
- Preparing responses to reviewers and corresponding with the editor
- Serving as the corresponding author

PUBLICATION 7

Liver Cirrhosis in Chronic Hepatitis B Patients Is Associated with Genetic Variations in DNA Repair Pathway Genes.

Rybicka M*, Woziwodzka A, Sznarkowska A, Romanowski T, Stalke P, Dręczewski M, Verrier ER, Baumert TF, Bielawski KP*.

Cancers (Basel). 2020 Nov 7;12(11):3295. doi: [10.3390/cancers12113295](https://doi.org/10.3390/cancers12113295)

*corresponding authors

IF=6,639; MEiN=140

Original article

My contribution to the publication included:

- Conceptualization and hypothesis development
- Development and optimization of the research methodology
- Leading role in planning and conducting experiments
- Leading role in collecting research material
- Creation of a clinical and pathological database of patients
- Interpretation and visualization of results
- Conducting statistical analyses of the obtained results
- Preparation of the initial draft of the manuscript, including tables and figures
- Revising the manuscript in response to journal reviewers' comments
- Preparing responses to reviewers and corresponding with the editor
- Serving as the corresponding author

PUBLICATION 8

Polymorphisms within DIO2 and GADD45A genes increase the risk of liver disease progression in chronic hepatitis b carriers.

Rybicka M*, Verrier ER, Baumert TF, Bielawski KP.

Scientific Reports 2023 Apr 14;13(1):6124. doi: [10.1038/s41598-023-32753-8](https://doi.org/10.1038/s41598-023-32753-8)

*corresponding author

IF=3,8; MEiN=140

Original article

My contribution to the publication included:

- Conceptualization and hypothesis development
- Developing and optimizing the research methodology
- Taking the leading role in planning and conducting experiments
- Taking the leading role in collecting research material
- Creating a clinical and pathological database of patients
- Interpreting the obtained results and their visualization
- Conducting statistical analyses of the obtained results

- Preparing the initial draft of the manuscript, including tables and figures
- Revising the manuscript in response to journal reviewers' comments
- Preparing responses to reviewers and corresponding with the editor
- Serving as the corresponding author

Scientometric data for the eight publications constituting the main habilitation achievement:

Total Impact Factor (IF) of the publications: 34.433

Total number of MEiN points according to the list for the year of publication: 665

Total 5-year Impact Factor (2024): 37.2

Current total number of MEiN points (2025): 830

4.3 Scientific aims of the works mentioned in point 4.2 and the obtained results

Introduction

Hepatitis B virus (HBV) infections are a global health problem, affecting approximately 296 million people with chronic infection. It is estimated that ~2 billion people have been exposed to the virus, meaning that nearly one in four individuals worldwide has been at risk of infection. In the countries of the European Union (EU) and the European Economic Area (EEA), 28,855 new cases of HBV infection were reported in 2022, the majority of which were chronic infections. It is estimated that about 3.6 million people in this region are living with chronic HBV infection, resulting in approximately 64,000 deaths annually. Globally, HBV causes around 820,000 deaths each year, and projections indicate a 39% increase in this number by 2030 (1,2).

Chronic hepatitis B (CHB) develops in 5–10% of infected adults, but in up to 90% of newly infected infants due to their immature immune systems. The key complications of HBV infection include liver cirrhosis, which develops in 20–30% of individuals with chronic infection and leads to approximately 331,000 deaths annually. Another major complication is hepatocellular carcinoma (HCC), which accounts for 57% of all HCC cases worldwide. The risk of developing HCC in individuals with CHB is 10–100 times higher compared to healthy individuals, especially in the presence of cirrhosis. One of the main risk factors for an increased frequency of complications is co-infection with the hepatitis D virus (HDV), which increases the risk of cirrhosis and HCC by four to five times compared to HBV mono-infection. In 2019, 192,000 people died from HBV-related HCC. Furthermore, HBV can result in acute (though rare) and chronic liver failure as a consequence of progressive fibrosis (1–3).

Despite the existence of an effective vaccine, which is the primary method of preventing hepatitis B, the fight against this disease faces a number of serious challenges. One of the main problems is insufficient prophylaxis. It turns out that only about 46% of newborns worldwide receive the first dose of the vaccine within the first 24 hours after birth. This is of particular concern in regions with high

endemicity, such as Southeast Asia and sub-Saharan Africa. Low global coverage of hepatitis B vaccination constitutes a significant obstacle to effective disease control. Many countries do not reach the WHO-recommended coverage level of 95%, which is crucial for effectively preventing the spread of infection. One of the important factors contributing to the low vaccination rate is misinformation about vaccines. The lack of reliable knowledge about the benefits of vaccination and misunderstanding of how vaccines work fosters vaccine hesitancy, both among the general public and sometimes among healthcare workers. Additionally, in some countries, the healthcare system is poorly organized, which hinders access to vaccines and other necessary medical services. Limited funding for vaccination programs in many regions also represents a serious barrier to achieving high vaccination coverage. An additional problem is the lack of access to anti-HBs immunoglobulin (targeted against the HBV surface antigen) in some regions, especially in developing countries. This immunoglobulin is an important element in preventing HBV transmission, and its absence makes newborns born to HBV-infected mothers more vulnerable to infection (1–4).

Another significant problem is the low availability of effective HBV diagnostic testing. Early detection of HBV infection is crucial for preventing complications such as cirrhosis and HCC. Unfortunately, only 14% of infected people are diagnosed, meaning that the majority of cases remain undetected. This is often due to the lack of symptoms in the acute phase of infection and limited access to diagnostic testing. Diagnosis of HBV infection is based on serological tests (Table 1), such as the detection of the hepatitis B surface antigen (HBsAg), as well as molecular tests that allow for the determination of HBV DNA levels in the blood. Rapid immunochromatographic tests, which enable the detection of HBsAg in a short time, are nowadays increasingly used and may help improve detection rates. Despite technological advances in diagnostics, access to HBV treatment remains limited. It is estimated that less than 10% of those infected receive appropriate therapy, which is a serious problem, especially in developing countries (5–7).

Table 1. The most important HBV markers and their clinical significance.

Marker	Clinical Significance
HBsAg	HBV surface antigen. Its presence indicates acute or chronic HBV infection. It is one of the first markers to appear after infection and persists throughout the period of active infection. Persistence of HBsAg for more than 6 months indicates chronic infection.
HBeAg	Secretory "e" antigen of HBV. A marker of intense viral replication and high infectivity. Its presence indicates an active phase of the disease and a high risk of HBV transmission. Persistence of HBeAg for more than 3 months suggests transition to the chronic phase.
anti-HBc IgM	Antibodies against the HBV core antigen, IgM class. They appear early in the acute phase of infection and persist for about 6 months. Their presence indicates a recent, acute infection.
anti-HBc total	Antibodies against the HBV core antigen (IgM + IgG). A marker of past contact with HBV or current infection. IgG persists for life.
anti-HBe	Antibodies against the HBe antigen. Their appearance indicates suppression of viral replication and transition of the infection to a less infectious phase. In chronic infection, the presence of anti-HBe usually means lower viral activity.
anti-HBs	Antibodies against the HBV surface antigen. Their presence indicates immunity to HBV (after vaccination or after recovery from infection).
HBV-DNA	Viral genetic material. The earliest marker of infection, allows quantitative assessment of viral replication activity and monitoring of treatment effectiveness.

The treatment of chronic hepatitis B (HBV) involves several key strategies aimed at inhibiting viral replication and improving patient health. One of the drugs used in HBV treatment is pegylated interferon- α 2a (Peg IFN α 2a), a cytokine that modulates the body's immune response. Another group of drugs consists of nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), among which lamivudine, telbivudine, and entecavir (nucleoside analogs), as well as adefovir and tenofovir (nucleotide analogs), are used in clinical practice. These drugs effectively suppress viral replication, reducing the risk of cirrhosis and hepatocellular carcinoma. Unfortunately, long-term therapy with NRTIs leads to the selection of drug-resistant strains and loss of therapeutic effect, which in patients with liver failure can result in organ damage or even death. Moreover, resistance-conferring mutations may occur in patients who have never been previously treated. An effective alternative to analog therapy is treatment with the aforementioned interferon, which does not cause the development of drug resistance. However, due to numerous side effects and contraindications, it cannot be used in all patients. Despite many advantages, the desired final effect of therapy depends on the viral genotype and is achieved in only about 30–40% of patients. The reason for the lack of treatment efficacy, unlike with NRTIs, is not the emergence of viral resistance to the drug, but most likely the interaction between the virus and immune system cells. The exact cause of interferon therapy failure has not yet been determined. Despite different mechanisms of action, none of the currently available drugs can effectively eliminate the virus, as they do not significantly affect covalently closed circular DNA

(cccDNA)—the key replicative reservoir of HBV that enables the production of new viral particles. Even a small number of cccDNA copies in hepatocytes can lead to reactivation of infection after discontinuation of treatment or weakening of immune control. For this reason, complete cure of chronic hepatitis B requires elimination of cccDNA. Due to insufficient knowledge of the HBV replication cycle, which results from the lack of an ideal experimental model, finding an optimal therapeutic regimen remains a major clinical challenge (8–11).

Research is currently underway on new drugs that may act on the stable form of cccDNA or prevent the virus from entering hepatocyte cells. The recent discovery of NTCP as the main receptor enabling HBV to enter hepatocytes, as well as the understanding of the role of host repair enzymes in the virus replication process, may help in the development of more effective treatment strategies. The 2022 guidelines of the European Association for the Study of the Liver (EASL) on the treatment of chronic HBV infection include recommendations on the use of interferons and NRTI. Reports from the World Health Organization (WHO) in 2024 emphasize the importance of early diagnosis and treatment in preventing HBV complications (9,12–16).

Description of the scientific purpose of the series of works and the results obtained.

The scientific objective of the presented series of publications was to identify viral factors and host genetic determinants that influence the clinical course and treatment response in chronic hepatitis B (HBV) infection. The research was based on the use of sensitive and modern molecular methods that enabled precise analysis of the HBV genome, including its cccDNA form, as well as selected regions of the human genome. In particular, the studies aimed to:

1. **Molecular characterization of the HBV genome**, with a focus on differences in the sequences of the relaxed circular form (rcDNA) and the covalently closed circular DNA form (cccDNA), which constitutes a persistent reservoir of the virus and is the main obstacle to complete elimination of the infection. (Publication 1, Publication 2)
2. **Identification and characterization of host genetic factors influencing the natural course of HBV infection, the risk of disease progression, and treatment efficacy.**

The research included the analysis of:

- genes encoding proteins involved in key stages of the virus life cycle (Publication 3, Publication 6)
- genes regulating the immune response (Publication 4, Publication 5)
- genes involved in DNA repair (Publication 7)
- genes involved in the regulation of cellular and adaptive responses (Publication 8)

3. **Integration of molecular data from the virus and host with clinicopathological information** to identify prognostic and predictive markers that can be used in clinical practice. (Publications: 2, 3, 4, 5, 7, 8)
4. **Development and optimization of sensitive research tools enabling** precise analysis of cccDNA sequences, analysis of host genetic polymorphisms, and integration of results using modern bioinformatic and statistical methods. (Publications: 2, 3, 4, 5, 7, 8)

The presented series of publications encompassed both basic and translational research, the results of which may be applied to the personalization of treatment, prognosis of disease course, and the development of new therapeutic strategies that integrate genetic information from both the virus and the host.

As part of the first stage of the research, the focus was on the review and evaluation of contemporary diagnostic methods used for the detection of the HBV virus. Publication 1, („**Current molecular methods for the detection of hepatitis B virus quasispecies**”, *Reviews in Medical Virology* 2016), constitutes a comprehensive analysis of the available molecular methods used for the detection and characterization of HBV quasi-species diversity and treatment-resistant variants. The article discusses both classical techniques (direct sequencing, restriction fragment length polymorphism – RFLP, single-strand conformation polymorphism – SSCP, hybridization techniques) as well as advanced high-sensitivity technologies, such as MALDI-TOF mass spectrometry, high-resolution melting analysis (HRM), and next-generation sequencing (NGS). Particular attention was given to detecting so-called minority variants (<5% of the viral population), which are often undetectable by standard diagnostic tests but may be responsible for treatment failure and the development of resistance. The advantages and limitations of each method were discussed in terms of their sensitivity, cost, reproducibility, and applicability in clinical diagnostics. The publication also highlights the need to introduce advanced diagnostic and monitoring tools into clinical practice, which is crucial for selecting therapy precisely tailored to viral mutations and for preventing complications of chronic hepatitis B. This publication serves as a bridge between basic research and its clinical application, aligning with the trend of translational research in the fields of diagnostics and biotechnology.

The second original publication („**Differences in sequences between HBV-relaxed circular DNA and covalently closed circular DNA**”, *Emerging Microbes & Infections* 2017), presents the results of studies comparing sequence differences between two forms of the HBV genome: the relaxed

circular form (rcDNA), present mainly in serum, and the covalently closed circular form (cccDNA), which constitutes a persistent reservoir of the virus in the nuclei of hepatocytes. The analysis was conducted on serum and liver biopsy samples collected simultaneously from 67 patients with chronic hepatitis B, treated at the Department of Infectious Diseases of the Medical University of Gdańsk and at the Hepatology Outpatient Clinic of the Pomeranian Center for Infectious Diseases and Tuberculosis in Gdańsk. In the first stage, a methodology was developed for the analysis of both forms of HBV DNA, including the relaxed circular DNA (rcDNA) and the covalently closed circular DNA (cccDNA). Experiments were also conducted to confirm the high sensitivity and specificity of the developed method for genotyping these DNA forms from clinical samples. Genotyping of the rcDNA form was performed directly after DNA isolation. For the analysis of the cccDNA form, two approaches were used. The first involved amplification of cccDNA using the rolling circle amplification (RCA) method, which mimics the natural replication of circular DNA and can be used to detect HBV cccDNA. The obtained products were then digested with the *SpeI* restriction enzyme, which cuts the HBV genome at a single site, linearizing it and allowing visualization of the full-length genome on an agarose gel. After separation of the digestion products on a 1% agarose gel, all bands corresponding to cccDNA were purified and analyzed by mass spectrometry. RCA amplification and *SpeI* digestion were performed under the same conditions as described by Margeridonet et al (17).

The second strategy involved removing the rcDNA form from the isolated DNA samples by using T5 exonuclease. This enzyme digests single-stranded DNA (ssDNA) in the 5' to 3' direction and is capable of removing nucleotides from the 5' end, both in ssDNA and at sites of nicks in the double-stranded structure of linear or circular dsDNA. Supercoiled cccDNA is resistant to digestion by T5 exonuclease because it lacks free 5' ends and nicks in its structure. To optimize the digestion of rcDNA, purified pAM6 plasmid (ATCC 45020D), containing a full-length HBV monomer, was used as a standard (Figure 1). The plasmid had previously been calibrated against the World Health Organization standard for HBV (HBV-A plasmid) and demonstrated the same amplification efficiency, which ensures the reliability of results obtained in molecular tests (18). In subsequent experiments, the selectivity of digestion by T5 exonuclease was demonstrated. Using the pAM6 plasmid, it was shown that T5 exonuclease does not damage the cccDNA form while effectively digesting a substantial excess (1 µg) of the linearized pAM6 plasmid, simulating rcDNA.

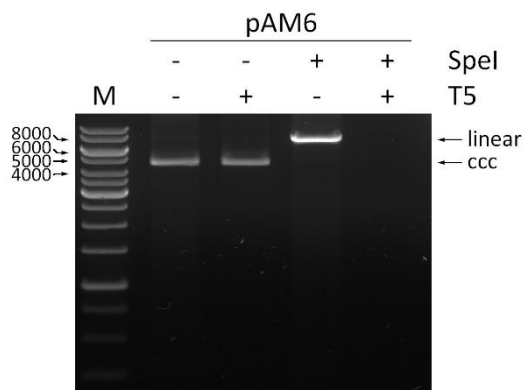


Figure 1. Assessment of T5 exonuclease digestion. In each case, 1 μg of the pAM6 plasmid (7.5 kb) was used. Plasmid linearization was performed using 10 units of Spel enzyme (NEB, Ipswich, MA, USA) at 37°C for 1 hour. Digestion with T5 exonuclease was carried out using 10 units of the enzyme (Epicentre) at 37°C for 30 minutes. The positions of linear and covalently closed circular (ccc) forms of the plasmid are indicated. Electrophoresis was performed in a 0.8% agarose gel in 1 \times TAE buffer. M denotes the DNA marker.

The next step was to compare the two previously described methods for detecting the cccDNA form of HBV. The RCA technique demonstrated low sensitivity—cccDNA was detected in only 1 out of 67 serum samples and in 48 out of 67 liver biopsy samples. Additionally, the results of this method did not correlate with clinical markers, suggesting the possibility of false-negative results and undermining its reliability. In contrast, the T5 exonuclease digestion method enabled the detection of cccDNA in all liver samples, while none was detected in serum samples, which is consistent with expectations for patients with chronic HBV infection. As a result, the method using T5 exonuclease was considered more reliable, sensitive, and suitable for cccDNA detection and was chosen as the technique for preparing cccDNA for genotyping.

After appropriate sample preparation, the sequences of the rc and cccDNA forms of the HBV virus were compared using the highly sensitive MALDI-TOF mass spectrometry technique. Mutations associated with drug resistance in the polymerase (pol) region, mutations affecting the host immune response in the precore (PC) region, and mutations in the basal core promoter (BCP) region—which can increase the rate of viral replication and also influence the virus’s phenotype or its sensitivity to treatment—were analyzed. The first step of the analysis involved amplification of selected HBV DNA fragments containing the mutations of interest. The resulting PCR products were then treated with SAP enzyme (shrimp alkaline phosphatase), which neutralizes unused dNTPs, thereby eliminating their impact on the accuracy of mass spectrometry readings in subsequent steps. The presence of HBV genetic variants was examined in four separate primer extension reactions using iPLEX Pro reagents (Agena Bioscience, USA). All primers were designed to anneal directly adjacent to the polymorphic site and were extended with the appropriate nucleotide depending on the template sequence. The resulting, variant-dependent differences in the mass of the extension products were detected directly using MALDI-TOF mass spectrometry.

By performing serial dilutions of the pAM6 plasmid, it was demonstrated that genotyping using the described method can be carried out unambiguously for template (plasmid DNA) amounts ranging from 1 μg to 10 ng per reaction, which corresponds to the cccDNA content found in clinical samples

(10–100 copies per hepatocyte). It was also shown that the addition of up to 10 ng of human genomic DNA does not interfere with the effective and unambiguous genotyping of pAM6 plasmid in amounts from 1 pg to 10 ng. In these experiments, 100% concordance with the reference sequence of the pAM6 plasmid was achieved.

The results of the conducted analysis have, for the first time, demonstrated that the rcDNA and cccDNA forms of HBV can have different nucleotide sequences. It was also proven that cccDNA is not present in the blood serum of individuals chronically infected with HBV. Another significant finding was the identification of sequence differences between the rcDNA form isolated from liver and blood samples taken from the same patient. These differences were observed in 39% of cases in the BCP/PC region and in 16% in the P region. This observation explains why the absence of mutations in plasma does not exclude the presence of clinically significant HBV mutations at levels below 1% in the hepatocytes of infected patients. These drug-resistant HBV variants, undetectable in circulation, are gradually eliminated from plasma but likely persist in hepatocytes—as a cellular reservoir of the virus's stable genetic material (cccDNA). In response to selective pressure, such as during antiviral therapy, relevant variants may reappear in the bloodstream. Importantly, although drug-resistant strains have lower replication potential, the presence of mutations in the BCP/PC region usually enhances their replication ability. Furthermore, the study detected differences between rcDNA and cccDNA sequences isolated from the same liver fragment, further supporting the hypothesis that HBV cccDNA acts as a reservoir for HBV mutations. It was also documented that mutations present in the cccDNA form have a significant impact on many clinical parameters in patients treated with interferon, such as the presence of iron in liver biopsy samples (mutation G1764A), the degree of liver inflammatory activity (G1764A, A1613T, G1899A), the degree of liver fibrosis (C1858T, A1762T, G1764A), and elevated liver enzyme levels (G1899A). In the case of rcDNA, only the G1613A mutation showed a correlation with the listed parameters, which further emphasizes the greater significance of mutations present in cccDNA. Based on the obtained results, it can be assumed that mutations in the cccDNA form are recorded during the HBV replication cycle and may quickly appear in plasma during active infection. Thus, the cccDNA form may serve as a “mutation reservoir” and be crucial for predicting the response to antiviral treatment, especially in previously treated patients. This work provides new data on the biological and therapeutic significance of the stable form of HBV DNA and may serve as a basis for the development of therapeutic strategies also targeting the viral reservoir.

The next objective of the presented series of publications was to define the relationships between host genetic polymorphisms and key clinical aspects of HBV infection, such as susceptibility to infection, viral load dynamics, risk of progression to advanced liver damage (fibrosis, cirrhosis, hepatocellular carcinoma - HCC), and the effectiveness of the response to antiviral therapy. These studies, conducted mainly in the European population, enabled the identification of new risk

biomarkers, deepened the understanding of HBV pathogenesis mechanisms, and indicated potential directions for therapy personalization.

As part of the conducted analyses, the focus was placed on single nucleotide polymorphisms (SNPs) in host genes that may be significant in the context of HBV infection. An assessment was carried out on 115 SNPs located in 49 host genes, such as: *cGAS*, *CRP*, *DDB1*, *ERCC2 (XPD)*, *ERCC5*, *FOXP3*, *FXR*, *GPC5*, *GPC5-AS1*, *HJURP*, *HNF1A*, *HNF4A*, *IFI6*, *IFNL4*, *IL10*, *IL12A*, *IL13*, *IL18*, *IL1B*, *IL28B*, *IL4*, *IL6*, *IL9*, *MBL*, *miR-196a2*, *miR-499*, *MRE11*, *MRE11a*, *NBN*, *Nbs1*, *NTCP*, *OGG1*, *POKL*, *PPAR α* , *RAD52*, *RRM2*, *STAT1*, *STAT2*, *STAT3*, *STING/TMEM173*, *TDP2*, *TIM-3/HAVCR2*, *TLR3*, *TLR9*, *TNF*, *TNFRSF10B*, *TRIM41*, *VDR*, *XRCC1*. The selection of genes was based on their potential involvement in the HBV life cycle within hepatocytes, in the host's immune response to infection, as well as in the indirect regulation of this response and the expression of viral genes, including the transcription of HBV proteins. The above study was conducted on genomic DNA isolated from the blood of patients.

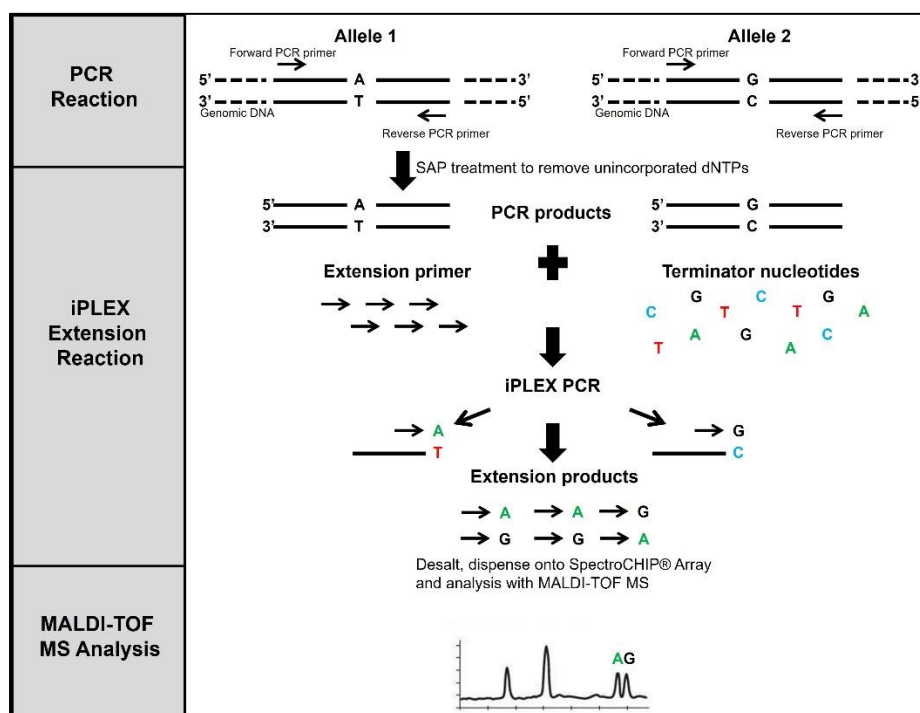


Figure 2. Analysis of SNPs by MALDI-TOF MS.

PCR: Amplification of selected DNA fragments containing the analyzed SNPs. The PCR products are then digested with the SAP enzyme (shrimp alkaline phosphatase), which neutralizes any unused dNTPs.

iPLEX PCR: Differentiating primers, designed directly adjacent to the SNP, as well as terminating nucleotides (ddNTPs), are added to the reaction. The primers are extended by one nucleotide corresponding to the allele present at the SNP site. The resulting

products, which differ by single nucleotides, are then purified on an ion-exchange resin, spotted onto a SpectroCHIP®, and subjected to a laser pulse to generate ions. Identification of the SNP variant is based on their mass-to-charge ratio.

Similarly to the earlier stages of the project, the MassARRAY® Analyzer platform (Agena Bioscience, USA), based on mass spectrometry technology, was used for genotyping. This method enables the simultaneous analysis of multiple SNPs in multiplex reactions with high sensitivity and accuracy, making it particularly useful in association studies (Figure 2). The analysis of the obtained data was performed using the Typer Analyzer Application v4 software (Agena Bioscience, USA).

Publication No. 3 (“**Host genetic background affects the course of infection and treatment response in patients with chronic hepatitis B**”, *Journal of Clinical Virology* 2019) served as the foundation for research into how genetic polymorphisms in host protein-coding genes influence HBV infection. This study focused on genetic variability in genes encoding proteins involved in the HBV replication cycle and the pathogenesis of chronic hepatitis B. The research examined *NTCP (SLC10A1)*, *FXR α (NR1H4)*, *HNF1 α* , *HNF4 α* , and *TDP2*, whose protein products play crucial roles at various stages of viral replication. The selection of SNPs in these genes was based on prior evidence indicating their potential functional significance. To assess the clinical relevance of the genetic variants under investigation, a well-defined group of 136 individuals diagnosed with chronic hepatitis B was studied. These participants were recruited from the Department of Infectious Diseases at the Medical University of Gdańsk and the Hepatology Clinic of the Pomeranian Center for Infectious Diseases and Tuberculosis in Gdańsk. The inclusion criteria for patients with hepatitis B required the persistence of HBsAg and anti-HBc IgG antibodies for at least 24 months prior to study enrollment. A diverse group of patients undergoing treatment was recruited, reflecting real-world clinical practice: Peg IFN α 2a (57%), lamivudine (29%), entecavir (12%), and tenofovir (2%). Additionally, detailed clinical and virological data were collected, including ALT (alanine aminotransferase), HBsAg, HBeAg, anti-HBe, quantitative HBV DNA measurement, and liver biopsy results (inflammatory activity and degree of fibrosis according to the Scheuer scale) (19). The control group consisted of 100 healthy individuals, all of whom were voluntary blood donors from the Blood Donation Center in Gdańsk.

Particular focus in this research was placed on examining the variability of the TDP2 gene, owing to its well-established role in the HBV replication cycle. The product of this gene—tyrosyl DNA phosphodiesterase 2—participates in the conversion of the viral genome from its relaxed circular (rcDNA) form to the stable covalently closed circular DNA (cccDNA), which serves as the main reservoir of HBV within cells and is a key factor in the persistence of chronic infection (20,21). Within the TDP2 gene, nine single nucleotide polymorphisms (SNPs) were examined. Of these, three (rs17249952, rs17249973, rs3212230) were found to be highly conserved in the study group. The remaining six SNPs were included in the statistical analysis: rs11559067 [T/C], rs1047782 [G/T], rs3087943 [A/G], rs707887 [C/A], rs1129644 [G/A], and rs2294689 [G/C]. A significantly higher frequency of the major allele (T) for rs11559067 was observed among women. Homozygosity for the major allele at rs1047782 [G/T] among patients treated with NRTI analogues was associated with an increased risk of severe hepatitis, suggesting a role for TDP2 in modulating the inflammatory response to chronic HBV infection. In contrast, individuals carrying the major allele at rs3087943 [A/G] had a significantly greater likelihood of achieving HBsAg seroconversion after Peg IFN α 2a treatment, which is considered the primary goal of antiviral therapy. This observation potentially links the role of TDP2 in cccDNA processing with the host’s ability to clear the virus during interferon therapy. Variability within the TDP2 gene, particularly

in regulatory regions such as the 3'UTR, may affect enzyme expression levels and, consequently, the efficiency of rcDNA to cccDNA conversion. This could in turn influence the intensity of viral replication, the degree of liver damage, and the overall effectiveness of antiviral treatment.

Another gene analyzed in this study was *NTCP* (*SLC10A1*), which encodes the functional HBV receptor on the surface of hepatocytes. This receptor allows the virus to enter cells by binding to the pre-S1 domain of the HBV envelope protein, making *NTCP* a key factor in determining cellular susceptibility to infection (22). Among the polymorphisms selected for analysis within the *NTCP* gene, only rs7154439 [A/G] exhibited variability in the study group. Patients with the AA genotype had a significantly higher likelihood of developing antibodies against the HBe antigen (anti-HBe) after 48 weeks of NRTI treatment. Thus, rs7154439 was associated with HBeAg seroconversion, which potentially implicates *NTCP* genetic variability in the host's ability to control HBV replication during analogue therapy. The results obtained suggest that rs7154439 may serve as a positive prognostic marker for viral elimination. Moreover, rs7154439 is located in the promoter region of the *NTCP* gene, which likely influences the expression of this protein by altering the binding affinity of transcription factors to the DNA sequence. The expression of *NTCP* is regulated by FXR α (NR1H4), a nuclear receptor activated by bile acids that controls both bile acid metabolism and *NTCP* levels. Additionally, FXR α can directly enhance HBV transcription by binding to response elements within the viral promoter region, leading to increased synthesis of pregenomic RNA and HBV DNA replication (23,24). No association was found in this study between the FXR α genotypes analyzed and any clinical or virological parameters.

Among analyzed genes, there were those encoding transcription factors (*HNF1A*, *HNF4A*), with *HNF1A* playing a particularly significant role. This gene is responsible for producing a protein that regulates the expression of numerous hepatic genes, including viral genes. HNF1 α exhibits antiviral activity—its overexpression inhibits HBV replication and protein expression by activating the NF- κ B signaling pathway. Conversely, a deficiency of this factor can lead to increased viral replication and higher levels of HBeAg antigen (25,26). Another key transcription factor is HNF4 α , which supports HBV replication and regulates the expression of its genome. Prolonged suppression of HNF4 α during chronic HBV infection promotes hepatocyte proliferation, which can lead to the development of hepatocellular carcinoma (HCC) (25,27). In the conducted studies, variability within the *HNF1A* gene was analyzed based on the following polymorphisms: rs1169288 [G/T], rs7310409 [A/G], and rs2464196 [G/A]. It has been demonstrated that the rs1169288TT genotype was associated both with a more advanced stage of liver fibrosis and with a higher likelihood of HBsAg seroconversion after 24 weeks of Peg IFN α 2a treatment. The rs7310409GG genotype was significantly associated with an increased probability of HBsAg elimination after completion of interferon therapy, whereas rs2464196AA correlated with the presence of HBV DNA after 48 weeks of treatment as well as with

greater severity of inflammatory changes and liver fibrosis. All SNP polymorphisms located in the *HNF1A* gene correlated with the presence of HBeAg antigen at the start of treatment and with the absence of anti-HBe antibodies in patients treated with NRTIs. Additionally, the rs7310409AA and rs2464196AA variants showed a significant association with the degree of liver inflammation, while rs2464196AA was also linked to the stage of liver fibrosis. These results indicate that polymorphisms in the *HNF1A* gene may influence the clinical course of HBV infection (degree of liver fibrosis, severity of inflammation) as well as the effectiveness of antiviral therapy (likelihood of HBsAg elimination, persistence of viremia). This confirms the role of HNF1A as a key regulator of HBV replication and the expression of genes essential for hepatocyte function. Genetic variability within this gene may serve as an important prognostic biomarker in antiviral therapy. In the study, two *HNF4A* variants—rs2144908 [G/A] i rs1800961 [C/T]—were also analyzed, with the latter showing an association with Peg IFN α 2a-induced HBsAg clearance during long-term follow-up. This observation suggests that HNF4 α may play a role in modulating the durability of the response to antiviral therapy, possibly by influencing the stability of viral antigen expression or hepatocyte metabolism

The study described in Publication No. 3 was the first that assessed the impact of polymorphisms in the *TDP2*, *NTCP*, *HNF1A*, *HNF4A*, and *FXR α* genes on the course of CHB and the effectiveness of treatment response in a European population. Special attention was given to the *NTCP* and *TDP2* genes, which are crucial for HBV entry into cells and the processing of cccDNA, highlighting their significant role in the pathogenesis of infection. The study demonstrated the influence of specific genetic variants on disease progression and therapy effectiveness, emphasizing the potential of personalized treatment approaches in the management of chronic hepatitis B. The results obtained justify further research in larger, well-defined cohorts to validate the identified associations and to investigate the underlying mechanisms by which these genetic variants affect HBV infection.

Expanding on this line of research, Publication No. 4 (**„*TNF- α* polymorphisms affect persistence and progression of HBV infection”**, *Molecular Genetics & Genomic Medicine* 2019) evaluated the impact of *TNF- α* gene polymorphisms on the risk of chronic HBV infection, the progression of liver damage, and the effectiveness of therapy. *TNF- α* (tumor necrosis factor alpha) is a key pro-inflammatory cytokine that plays a crucial role in the immune response against HBV—both by supporting viral elimination and by regulating inflammation in the liver (28). In the study, five single nucleotide polymorphisms (SNPs) from the promoter region of the *TNF- α* gene, previously recognized as important in the regulation of cytokine expression, were analyzed: -1031T/C (rs1799964), -863C/A (rs1800630), -857C/T (rs1799724), -308G/A (rs1800629), and -238G/A (rs361525). The study hypothesized that genetic variants of *TNF- α* may modulate the immune response against HBV, thereby affecting the level of viral replication and the progression of liver disease. Compared to Publication No.

3, the study significantly increased the number of participants—to 231 individuals, representing a 70% rise over the previous sample (136 patients). This expansion contributed to improved statistical power and reliability of the results. The analysis was also deepened to include a broader range of parameters, such as the presence of specific *TNF- α* gene haplotypes and the relationship between viremia levels and genotype. Diagnosis of hepatitis B was based on the presence of HBsAg and anti-HBc IgG for at least 24 months. All patients were of European origin and came from northern Poland. Additionally, as many as 196 individuals underwent liver biopsy, enabling assessment of the degree of inflammation and fibrosis according to the Scheuer scale (19). A parallel group of 100 healthy blood donors was recruited as a local control group—these were also adult Europeans, seronegative for HIV (human immunodeficiency virus), HBV, and HCV (hepatitis C virus). Both groups underwent genotyping for five polymorphisms in the promoter region of the *TNF- α* gene using MALDI-TOF mass spectrometry. Haplotype analysis was performed with the SNPstats tool ([SNPStats: your web tool for SNP analysis.](#)), revealing the presence of five distinct *TNF- α* variants. The most common haplotype was TGGCC (covering positions -1031, -238, -308, -863, and -857), which was found in over half of the individuals studied. Haplotype frequencies were similar between HBV-infected patients and healthy controls. However, the -1031C and -863A alleles appeared more frequently in the group of patients with hepatitis B than in the control group, suggesting their association with increased susceptibility to chronic HBV infection. Additionally, variability at these positions correlated with HBV DNA levels, indicating their potential significance in the regulation of viral replication (Figure 3).

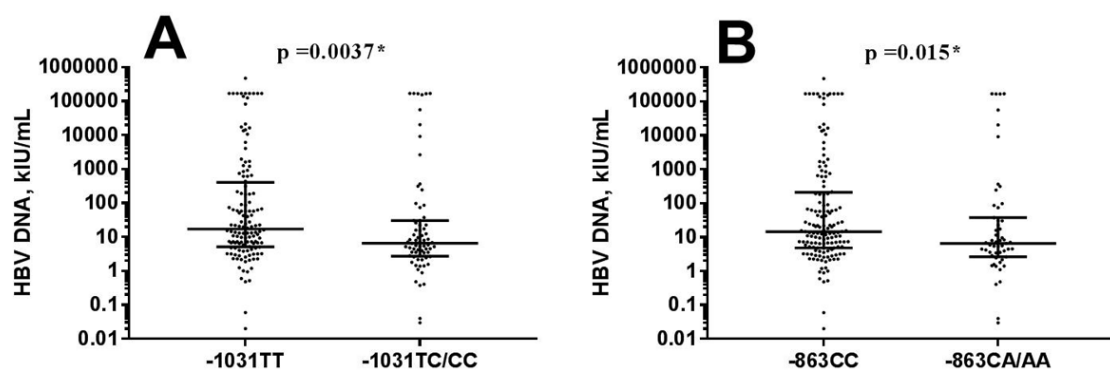


Figure 3. *TNF- α* promoter polymorphisms: -1031T/C (rs1799964) (A), -863C/A (rs1800630) (B) and baseline HBV DNA levels in patients with chronic hepatitis B. Lines represent median values and interquartile ranges. $p < .05$ are marked in bold. * p values remained significant after the adjustment for multiple testing.

Individuals with the -1031TT genotype had significantly higher serum HBV DNA levels compared to carriers of the C allele. A similar relationship was observed for the -863C/A polymorphism, where individuals with the CC genotype exhibited higher viremia than those carrying the A allele, in both genetic analysis models. Thus, carriers of the rarer alleles at both positions were characterized by lower concentrations of HBV DNA in serum.

Among the analyzed polymorphisms, $-1031T/C$, $-863C/A$, and $-857C/T$ were found to influence necroinflammatory activity in the liver (Figure 4). The $-1031TT$ (rs1799964TT) and $-857CC$ (rs1800630CC) genotypes were detected more frequently in patients with markedly increased liver inflammation. The $-857CT$ genotype was also associated with higher necroinflammatory activity in the liver. However, none of the studied *TNF- α* polymorphisms showed any effect on the progression of liver fibrosis, both in unadjusted analyses and after accounting for demographic variables such as sex and age of the patients

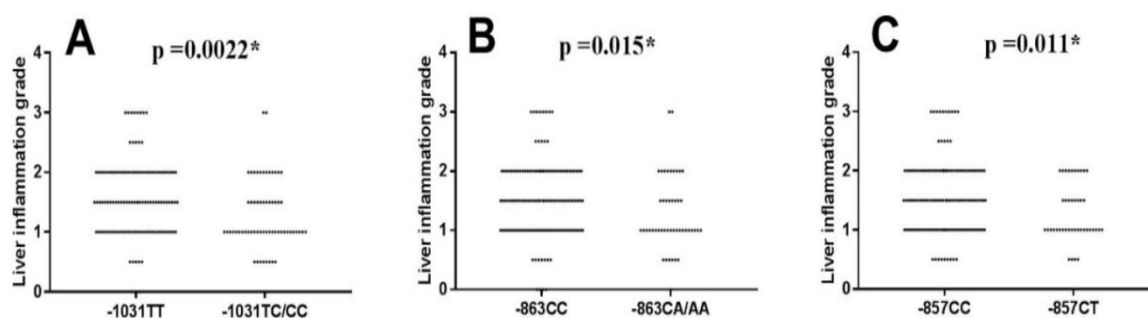


Figure 4. *TNF- α* promoter polymorphisms: $-1031T/C$ (A), $-863C/A$ (B), $-857C/T$ (C) and liver necroinflammatory activity in patients with chronic hepatitis B. $p < .05$ are marked in bold. * p values remained significant after the adjustment for multiple testing.

In summary, the conducted study confirmed the role of *TNF- α* in the pathogenesis of chronic hepatitis B, indicating an association between gene polymorphisms and both the risk of chronic infection and the dynamics and severity of liver damage during HBV infection. The research demonstrated the existence of variants that favor the persistence of infection ($-1031CC$ and $-863AA$), which are also linked to lower levels of viremia and a milder course of inflammation. In contrast, genotypes potentially beneficial in terms of the risk of chronic hepatitis B ($-1031TT$ and $-857CC$) were associated with a more aggressive immune response and intensified inflammation, which may accelerate the progression of liver disease. *TNF- α* , as a major pro-inflammatory cytokine, plays an important role in fighting HBV by limiting its replication. However, excessive or uncontrolled activation of *TNF- α* can damage hepatocytes. This explains the association of the $-1031TT$ (rs1799964TT) and $-857CC$ (rs1800630CC) variants with higher inflammation and a more aggressive disease course.

Publication No. 5 („Genetic variation in *IL-10* influences the progression of hepatitis B infection”, *Journal of Infectious Diseases* 2019) regarding *IL-10* gene polymorphisms represents an important addition to research on the genetic determinants of the host immune response in HBV infection. Interleukin 10 is a cytokine that acts antagonistically to pro-inflammatory cytokines such as *TNF- α* . While *TNF- α* intensifies the inflammatory response and directly inhibits viral replication, *IL-10* exerts immunosuppressive effects. Although this function helps to limit liver damage resulting from

excessive inflammatory response, it can also reduce the ability to clear the virus from the body, thereby increasing the risk of chronic infection (29–32).

A large cohort of patients was assembled for the study of *IL-10* polymorphisms. The analysis included 857 individuals with CHB and 48 patients with functional cure of infection (HBsAg-negative and anti-HBc-positive), allowing for the assessment of the impact of genetic variants both on the course of chronic infection and on the possibility of its spontaneous resolution. Samples from 648 study participants were obtained through international collaboration with the French partner, Professor Thomas Baumert from INSERM in Strasbourg, following prior approval of the application by the French Hepather committee. This collaboration enabled a significant expansion of the database and increased the clinical and geographic diversity of the analyzed population. Additionally, as in the study on *TNF- α* , a local control group was included—100 healthy blood donors, seronegative for HIV, HBV, and HCV.

The study assessed whether polymorphisms in the interleukin 10 (*IL10*) gene influence the response to antiviral therapy (both Peg IFN α 2a and NRTIs), as well as the progression of the disease to liver damage, including cirrhosis and hepatocellular carcinoma (HCC). Liver biopsy samples were evaluated for inflammatory activity and the degree of fibrosis according to the Scheuer scoring system (19). For individuals from whom a biopsy was not taken, liver status was assessed using FibroScan — a non-invasive, painless ultrasound method that measures liver stiffness and the degree of fibrosis, with results converted to the METAVIR scale(33) The assessment of liver status was further expanded by using noninvasive indicators such as the FIB-4 index ($[\text{age} \times \text{AST}] / [\text{platelets} \times \sqrt{\text{ALT}}]$), where values >3.25 indicate advanced fibrosis, and the APRI index ($[\text{AST} / \text{upper limit of normal}] / \text{platelets} \times 100$), where a result >2.0 suggests cirrhosis. This approach allowed for the supplementation of biopsy data and enabled a more comprehensive evaluation of the degree of fibrosis and inflammatory activity in the liver (34).

Following *IL10* variants were analyzed: rs1800872 (–592C/A), rs1800871 (–819C/T), rs1800896 (–1082G/A), rs1800893 (–1353C/T), rs3024490 (+504G/T), and rs1518110 (+954G/T). The association of *IL10* genotypes and haplotypes with clinical and virological data—such as HBV viremia, HBeAg seroconversion, HBsAg concentration, ALT/AST activity, degree of liver fibrosis (FIB-4 score, APRI), and response to treatment (Peg IFN α 2a and NRTIs)—was evaluated.

This study demonstrated that the rs1800896AA and rs1800893CC genotypes in the *IL-10* gene are more frequently found in patients with lower serum HBV DNA levels (<2000 IU/ml). Statistical analysis showed that individuals with the AA genotypes (rs1800896) had nearly twice the odds of having lower viremia compared to carriers of the GG or GA genotype. A similar trend was observed for rs1800893CC. This relationship is explained by the fact that polymorphisms in the *IL10* promoter region (rs1800896, rs1800893) influence interleukin 10 expression, which, as an immunosuppressive

cytokine, inhibits the inflammatory response. Therefore, lower IL-10 production in carriers of the CC genotypes may promote more effective control of HBV replication.

Furthermore, the analyzed *IL-10* polymorphisms were associated with therapeutic response in chronic hepatitis B, which is particularly important due to the immunomodulatory role of this cytokine. Treatment efficacy was assessed based on the loss of HBsAg, a key indicator of functional cure that is linked to the elimination of the viral reservoir (cccDNA) and a reduction in the risk of cirrhosis and the development of HCC. Three of the analyzed polymorphisms within *IL10* were significantly associated with achieving HBsAg seroclearance 24 weeks after the completion of Peg IFN α 2a therapy. Higher frequencies of minor alleles in patients who achieved HBsAg loss were observed at the following positions: rs1800871 (-819T), rs1800872 (-592A), and rs3024490 (+504T). In the group of patients treated with NRTIs, only the rs1800896 polymorphism showed an association with HBsAg loss; however, statistical significance disappeared after Bonferroni correction. In contrast, the *IL10* ATAC haplotype (1082A / 819T / 592A / 1353C) increased the likelihood of achieving the HBsAg-negative phase regardless of the type of therapy used, highlighting its key role in modulating the immune response in patients with chronic hepatitis B. Importantly, all six *IL10* polymorphisms analyzed showed statistically significant differences in the distribution of genotypes and/or alleles between patients who achieved HBsAg loss and those who did not experience seroclearance (Table 2).

SNP ID	Genotype	Genotypic distribution (%)			Allele	Allelic distribution (%)		
		HBsAg (+) (n=320)	HBsAg (-) (n=24)	<i>P</i> value		HBsAg (+) (n=640)	HBsAg (-) (n=48)	<i>P</i> value
-592C/A (rs1800872)	CC	185 (58)	6 (25)	0.0007	C	471 (74)	22 (46)	0.000039
	CA	101 (31)	10 (42)		A	169 (26)	26 (54)	
	AA	34 (11)	8 (33)					
-819C/T (rs1800871)	CC	182 (57)	6 (25)	0.0005	C	470 (73.5)	22 (46)	0.000044
	CT	106 (33)	10 (42)		T	170 (26.5)	26 (54)	
	TT	32 (10)	8 (33)					
-1082G/A (rs1800896)	GG	61 (19)	1 (4)	0.03	G	263 (41)	10 (21)	0.005653
	GA	141 (44)	8 (33)		A	377 (59)	38 (79)	
	AA	118 (37)	15 (63)					
-1353C/T (rs1800893)	CC	122 (38)	14 (58)	0.041	C	363 (57)	37 (77)	0.005809
	CT	119 (37)	9 (38)		T	277 (43)	11 (23)	
	TT	79 (25)	1 (4)					
+504G/T (rs3024490)	GG	173 (54)	7 (29)	0.005	G	462 (72)	24 (50)	0.001132
	GT	116 (36.5)	10 (42)		T	178 (28)	24 (50)	
	TT	31 (9.5)	7 (29)					
+954 G/T (rs1518110)	GG	188 (59)	12 (50)	0.0021	G	476 (74.5)	28 (58)	0.015446
	GT	100 (31)	4 (17)		T	164 (25.5)	20 (42)	
	TT	32 (10)	8 (33)					

HBsAg (+) – HbsAg positive patients; HBsAg (-) – HbsAg negative patients; *P* values less than 0.05 are marked in bold. All *P* values remained significant after Bonferroni correction.

Table 2. Genotypic and allelic distribution of analyzed *IL10* polymorphisms between CHB patients (HBsAg positive) and individuals who achieved HBsAg loss induced by antiviral treatment (HBsAg negative) in a 48-week follow-up.

To assess the degree of liver damage in the studied population, logistic regression analysis was performed based on FIB-4 and APRI indices, taking into account variables such as *IL10* genotypes, sex, and age. In the multivariate analysis, the rs1800871TT, rs1518110TT, rs1800872AA, and rs3024490TT genotypes were identified as predictors of lower FIB-4 scores ($p < 0.05$), indicating less severe liver fibrosis (Table 3). Similar observations were noted for the APRI index, whose values were significantly lower in patients homozygous for the minor allele in the case of these four *IL10* gene polymorphisms. The *IL10* ATAC haplotype (1082A / 819T / 592A / 1353C) also played a significant role in the analysis, occurring much more frequently in patients with milder liver damage. Conversely, the GCCT haplotype (1082G / 819C / 592C / 1353T) increased the risk of developing liver cirrhosis.

Tabela 3. Relationship between *IL10* polymorphisms and severity of liver disease in HBV infected patient.

<i>IL10</i> genotype	MAF	Odds ratio	95% CI	<i>P</i> value
rs1800871 [C/T]				
CC,CT vs. TT	T=0.43	8.94	1.78-44.80	0.007*
rs1800872 [C/A]				
CC,CA vs. AA	A=0.39	9.15	1.82-45.89	0.006*
rs3024490 [G/T]				
GG,GT vs. TT	T=0.43	9.02	1.79-45	0.007*
Rs1518110 [G/T]				
GG,GT vs. TT	T= 0.42	8.84	1.76-44.29	0.008*

Liver fibrosis was assessed by the *FIB-4* index. MAF – minor allele frequency. *P* values marked with * remained significant after Bonferroni correction

The results obtained indicate that differences in the *IL10* gene promoter sequence can influence both the rate of liver fibrosis progression and the response to antiviral treatment. Individuals with variants associated with lower IL-10 expression more frequently achieve HBsAg elimination following interferon or nucleos(t)ide analogue therapy and are also less likely to develop advanced fibrosis or cirrhosis. In contrast, variants linked to higher IL-10 production increase the risk of chronic infection and progression of liver damage, due to the immunosuppressive effect of this cytokine—it inhibits the inflammatory response but may also reduce the effectiveness of viral clearance by the immune system. In practice, this means that analyzing *IL-10* polymorphisms can help identify patients who are particularly at risk for a chronic course of disease and hepatic complications, as well as those with a greater chance of a successful therapeutic response. Thus, *IL-10* polymorphisms may serve as prognostic markers, supporting the personalization of treatment and monitoring the risk of fibrosis and cirrhosis progression in individuals with chronic hepatitis B.

As part of further investigations into factors influencing the progression of liver diseases induced by HBV, the variability of genes responsible for DNA repair was analyzed for their impact on the risk of developing cirrhosis in patients with chronic hepatitis B. Publication No. 7 („**Liver Cirrhosis in Chronic Hepatitis B Patients Is Associated with Genetic Variations in DNA Repair Pathway Genes**”, *Cancers* 2020) focuses on cirrhosis as the final stage of chronic liver diseases, which represents a significant global health issue and is the main risk factor for the development of hepatocellular carcinoma. The research assessed the frequency and significance of genetic variants in key DNA repair genes, such as *XRCC1*, *XRCC4*, *ERCC2*, *ERCC5*, *RAD52*, *Mre11*, and *NBN*. The selection of genes for analysis was based on their crucial roles in the main DNA repair pathways and their documented associations with HBV replication and the pathogenesis of liver cirrhosis. The study group consisted of 752 individuals of Caucasian origin, including 652 patients with chronic hepatitis B and 100 healthy blood donors as a control group. Patients were recruited both in Poland (Gdańsk, Gdynia) and in France (Strasbourg), reflecting the international collaboration involved in conducting the study. Only individuals who were either treatment-naïve or had received antiviral therapy for a short period (<6 months) were included in the study. The degree of liver fibrosis was assessed at the time of cohort enrollment. In 132 patients, a liver biopsy was performed, and the severity of fibrosis as well as inflammatory activity were evaluated according to the Scheuer scoring system (19). In the remaining 520 patients who did not undergo biopsy, noninvasive transient elastography (FibroScan) was used to assess liver fibrosis. Fibrosis and cirrhosis were classified according to the METAVIR scoring system (33).

Significant differences were observed in the distribution of genotypes of the six analyzed polymorphisms (*ERCC2*: rs13181, rs238406; *RAD52*: rs7963551; *XRCC1*: rs25487; *ERCC5*: rs2018836; *NBN*: rs2735383) between patients with chronic hepatitis B and healthy individuals from the control group, suggesting their association with susceptibility to chronic HBV infection. In addition, the *ERCC2* rs238406GG, *XRCC1* rs25487TT, and *NBN* rs2735383GG genotypes were identified more frequently in patients with cirrhosis than in patients without fibrosis (Figure 5). This observation highlights the role of the analyzed variants in modulating the risk of cirrhosis in patients with chronic hepatitis B, regardless of classic clinical factors.

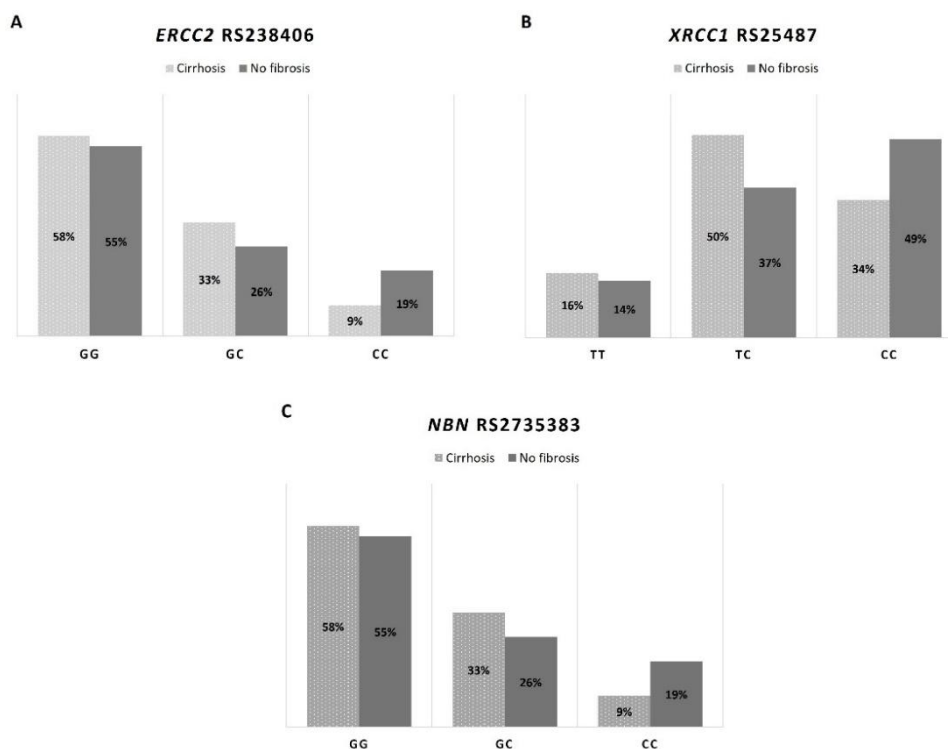


Figure 5. Distribution of *ERCC2* rs238406 (A), *XRCC1* rs25487 (B), and *NBN* rs2735383 (C) genotypes in patients with liver cirrhosis and in patients without signs of liver fibrosis.

In the study group rs13181TT (*ERCC2*) and rs2018836GG (*ERCC5*) genotypes were more common in patients with lower HBV DNA levels, and the rs25487TT (*XRCC1*) genotype was associated with lower liver enzyme levels (ALT and AST). In addition, carriers of rs2735383CC (*NBN*) had significantly lower ALT levels.

To investigate the association between DNA repair gene polymorphisms and liver cirrhosis in patients with CHB, three genetic models were used: the additive model (analyzes the linear effect of each allele copy on the phenotype), the dominant model (compares the presence of at least one risk allele, for example: GT+TT vs. GG); and the recessive model (assesses the effect of homozygosity for the risk allele, for example: TT vs. GT+GG). The study employed a two-step statistical analysis. In the first step, univariate analyses were conducted, which allowed the identification of clinical and genetic factors associated with the risk of developing liver cirrhosis. Subsequently, these results were verified in a multivariate model that accounted for confounding variables such as age, sex, and laboratory parameters. It was found that the rs238406TT genotype in the *ERCC2* gene and the rs25487CC genotype in the *XRCC1* gene significantly reduce the risk of developing cirrhosis. A similar analysis was conducted to examine the relationship between the evaluated genetic variants and the degree of liver fibrosis. The rs238406TT (*ERCC2*) and rs25487CC (*XRCC1*) genotypes were identified as independent predictors of a lower degree of fibrosis.

The results highlight that individual differences in DNA repair efficiency modulate the progression of fibrosis in patients with chronic hepatitis B. ERCC2 participates in the nucleotide excision repair (NER) pathway, and its effectiveness is important for protecting the genome against mutations that can lead to, among other things, cancer. Variants of *ERCC2* (e.g., the G allele of rs238406) may be associated with impaired repair, which can promote the integration of HBV DNA into the host genome, activating pro-inflammatory pathways and fibrosis. In contrast, *XRCC1* coordinates the repair of single-strand DNA breaks caused by reactive oxygen species (ROS), whose production increases during chronic hepatitis. Carriers of the T allele (rs25487) have reduced base excision repair (BER) capacity, leading to the accumulation of mutations in hepatocytes and accelerated fibrosis progression. Additionally, since HBV utilizes DNA repair pathway proteins—among other functions—to convert rcDNA into cccDNA, polymorphisms in DNA repair genes (such as *ERCC2* and *XRCC1*) may modulate the efficiency of this process, affecting viral replication levels and the extent of liver damage. The findings indicate the potential use of genetic profiling of DNA repair genes to identify hepatitis B patients at increased risk of developing cirrhosis, which would allow for the early implementation of appropriate therapeutic strategies. Patients with risk alleles (e.g., *ERCC2* rs238406G, *XRCC1* rs25487T) should undergo more frequent monitoring of fibrosis parameters (such as elastography and FibroTest). Detection of these variants may support decisions regarding earlier initiation of antiviral therapy or the use of antifibrotic drugs.

In publication no. 8 („**Polymorphisms within *DIO2* and *GADD45A* genes increase the risk of liver disease progression in chronic hepatitis B carriers**”, *Scientific Reports* 2023) the scope of research on chronic hepatitis B was broadened to encompass the full spectrum of liver disease progression—from the absence of fibrosis, through mild and moderate forms, to cirrhosis and hepatocellular carcinoma (HCC). Genes associated with the regulation of cellular stress response (*GADD45A*, *ATF3*), control of metabolism and metabolic adaptation (*DIO2*, *PPARG*), as well as modulation of the immune response (*TBX21*, *AKT3*) were studied. A total of 284 patients with confirmed chronic hepatitis B infection—defined as the presence of HBsAg for more than six months—were included in the study. All participants were drawn from the ANRS CO22 HEPATHER cohort (ClinicalTrials.gov: NCT01953458). The entire study group consisted of individuals of European origin who had no other coexisting causes of liver disease, such as viral coinfections, autoimmune disorders, or metabolic diseases. Patients who were undergoing antiviral therapy or had completed such treatment within six months prior to the start of the study were excluded. Serum samples for laboratory testing were collected before the assessment of liver fibrosis, with analyses including serological markers related to HBV (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and HBV DNA levels). The degree of fibrosis was assessed using non-invasive FibroScan transient elastography and patients were classified according to the METAVIR system (33).

Participants were divided into groups: without fibrosis (stage F0), with mild fibrosis (stage F1), with moderate to severe fibrosis (stages F2–F3), and with liver cirrhosis (stage F4, confirmed by two experienced pathologists). Genotyping of SNP polymorphisms in the *DIO2*, *PPARG*, *ATF3*, *AKT3*, *GADD45A*, and *TBX21* genes was performed using MALDI-TOF mass spectrometry. To identify independent risk factors for fibrosis progression, development of cirrhosis, and HCC, dominant and allelic models were used. To assess the functional impact of the analyzed polymorphisms, bioinformatic tools were used that enabled the prediction of their potential effects on protein structure, stability, and function, as well as on the regulation of gene expression (Table 4). The application of these tools allowed for a multidimensional analysis of the genetic variants' consequences, both at the protein and regulatory levels, which provided a better understanding of their role in the progression of liver diseases in patients with chronic hepatitis B.

Table 4. Bioinformatics tools applied for the functional evaluation of the analyzed polymorphisms.

Name	Application	Notes
SIFT	Predicting the impact of SNP polymorphisms on protein function based on sequence homology and physicochemical properties of amino acids	Scale 0–1, where a score ≤ 0.05 indicates a deleterious effect; considers sequence homology and amino acid physicochemical properties
MUpro	Assessing the impact of mutations on protein structural stability ($\Delta\Delta G$)	$\Delta\Delta G$ value < 0 suggests protein destabilization; tool uses machine learning algorithms
I-Mutant 3.0	Analysis of protein stability changes after mutation ($\Delta\Delta G$)	Estimates whether a mutation leads to protein destabilization or stabilization
HOPE	Analysis of structural and functional effects of mutations	Visualizes changes in protein structure, such as size, hydrophobicity, potential disruption of functional domains
MetaRNN	Prediction of SNP pathogenicity	Scale 0–1, where a higher value indicates a greater probability of a pathogenic effect; incorporates multiple genomic features
RegulomeDB	Assessment of regulatory potential of SNPs in non-coding regions	Scale 0–1; 1 indicates the highest probability of regulatory function; considers TF binding, chromatin states
miRNASNP	Prediction of the impact of SNPs on the loss or gain of miRNA binding sites	Assesses the effect of variants on post-transcriptional gene expression regulation by microRNAs

SIFT (https://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html); MUpro (<http://mupro.proteomics.ics.uci.edu/>); HOPE (<https://www3.cmbi.umcn.nl/hope/>);

I-Mutant 3.0 (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi>); miRNASNP (<https://guolab.wchscu.cn/miRNASNP/#/1/>);

MetaRNN (www.jiulab.science/metarnn.html); RegulomeDB (<https://beta.regulomedb.org/regulome-search/>); HOPE (<https://www3.cmbi.umcn.nl/hope/>);

Polymorphisms in the *DIO2*, *AKT3* i *GADD45A* genes showed significant associations with clinical and virological features in patients with chronic hepatitis B. The *DIO2* genotypes (rs225014CC and rs225017TT) were more frequently observed in men, and the presence of HBeAg was more common among carriers of rs225014CC. The *AKT3* rs12031994TT genotype was associated with higher HBsAg levels, whereas rs12031994CC was linked to elevated AST levels. In multivariate analysis,

independent predictors of higher ALT included thrombocytopenia, *DIO2* rs225014TT, *AKT3* rs12031994TT, and *GADD45A* rs532446CC.

The association between polymorphisms and the progression of liver fibrosis was also evaluated. The *DIO2* rs225014TT genotype was significantly less frequent in the F0 group than in the F1–F4 groups. Differences were also noted for rs10865710 (*PPARG*) and rs4794067 (*TBX21*) (Figure 6). In further multivariate analysis, the rs225014TT genotype (*DIO2*), rs10865710CC genotype (*PPARG*), and the presence of portal hypertension remained independent predictors of advanced fibrosis.

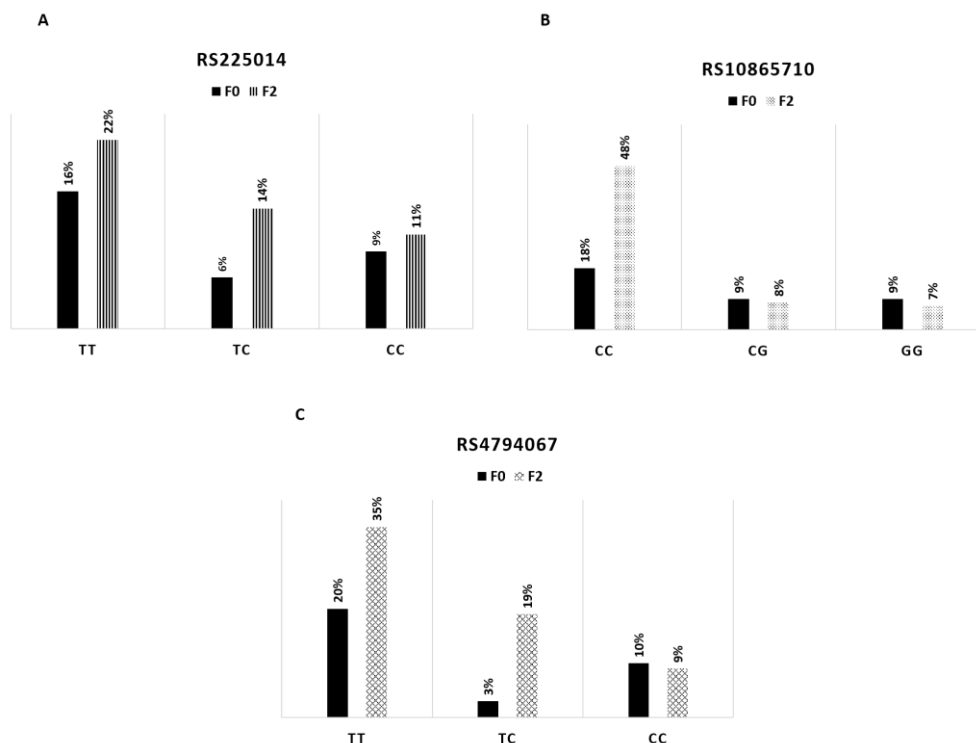


Figure 6. Graphs showing genotype distribution of *DIO2* rs225014 (A), *PPARG* rs10865710 (B) oraz *TBX21* rs4794067 (C) in patients with fibrosis stage F0 and F2.

Similar analysis was conducted to determine the relationship between the studied genetic variants and liver cirrhosis. In the multivariate analysis, after accounting for confounding variables, the following were identified as independent risk factors for liver cirrhosis: thrombocytopenia, elevated ALT levels, rs532446TT (*GADD45A*), and rs11119982TT (*ATF3*).

Hepatocellular carcinoma (HCC) was detected in 13 out of 284 (4.6%) patients with chronic hepatitis B. No direct association was found between the analyzed SNPs and the presence of HCC in the study group. However, a different distribution of *DIO2* rs225014 genotypes was observed between patients with cirrhosis who developed HCC and those without cancer. The rs225014CC variant was identified in 38% of patients with HCC compared to 12% of patients with cirrhosis without HCC.

In silico analysis found that the polymorphisms rs225014 (*DIO2*), rs10865710 (*PPARG*), rs532446 (*GADD45A*), and rs4794067 (*TBX21*) exhibit the strongest features suggesting their potential regulatory function. The rs10865710 polymorphism has been associated with the regulation of *PPARG* and *TIMP4* gene expression, as well as with the ability to modulate the binding of the JUN protein and transcription factors from the NFATC family. It has been demonstrated that rs532446 is located within ATF4 and PRDM binding motifs and interacts with multiple regulatory proteins, whereas rs225014 modulates the expression of target genes and the binding of transcription factors. Rs4794067 was associated with the expression of numerous genes and interactions with the EZH2 and CTCF proteins. Histone modification analysis revealed that rs10865710, rs532446, and rs4794067 are located within DNA regions marked as transcriptional enhancers in liver tissue, as well as in endocrine and exocrine glands. This suggests their role in regulating the expression of genes crucial for metabolic and immune functions. Additionally, the miRNASNP tool demonstrated that all of the aforementioned SNPs can modify microRNA binding sites (e.g., miR-3143, miR-4488), thereby affecting the post-transcriptional regulation of gene expression.

This study provides groundbreaking data on the genetic determinants of chronic hepatitis B progression, identifying specific SNP polymorphisms (rs225014 in *DIO2*, rs532446 in *GADD45A*, rs10865710 in *PPARG*) that significantly correlate with the risk of advanced fibrosis, cirrhosis, and HCC. For example, the rs532446 TT genotype (*GADD45A*) increases the risk of cirrhosis by 4.8 times, confirming the crucial role of cellular stress response pathways in the pathogenesis of the disease. The analysis of molecular mechanisms has shown that destabilization of the *DIO2* protein disrupts thyroid hormone metabolism, leading to oxidative stress and hepatocyte damage. Meanwhile, polymorphisms in the *PPARG* and *TBX21* genes modify metabolic and immune pathways.

These results have direct implications for personalized medicine. Genotyping of the indicated SNPs enables the identification of high-risk patients who require more intensive supervision (e.g., more frequent imaging studies for HCC). At the same time, the study points to new therapeutic targets, such as inhibition of the *GADD45A/ATF3* pathway or modulation of *PPARG* activity, which could form the basis for the development of innovative drugs that slow disease progression. Integrating genetic data with classical clinical parameters opens the door to more accurate diagnostic and therapeutic algorithms, significantly improving the prognosis for patients with chronic hepatitis B.

Publication No. 6 („**Recent Advances in Understanding, Diagnosing, and Treating Hepatitis B Virus Infection**”, *Microorganisms* 2020) provides a comprehensive overview of research topics related to HBV infection, including its diagnosis, pathogenesis and modern therapeutic strategies. This review article covers the current state of knowledge regarding the epidemiology, molecular biology and life cycle of the HBV virus. The article discusses the latest advances in diagnostics, paying particular

attention to new viral biomarkers such as HBV RNA, HBcrAg, anti-HBs and anti-HBc antibodies. The article also addresses the risk of progression to advanced liver damage (fibrosis, cirrhosis, HCC) and the effectiveness of antiviral therapy, both in the context of traditional drugs (interferon, NRTIs) and promising new therapeutic strategies targeting various stages of the HBV replication cycle, including cccDNA formation. The publication integrates and summarizes research findings on host factors, their impact on the course of infection, and response to treatment, which corresponds to the scope of studies presented in the habilitation application. The article presents an interdisciplinary perspective on HBV issues, combining molecular, genetic, clinical, and translational aspects, situating the authors' own observations within the broad context of international literature.

The publications presented in the habilitation application focus on the molecular and genetic aspects of chronic hepatitis B virus (HBV) infection, with a particular attention to the biotechnological dimension of the research. The articles, published in internationally recognized journals, make a significant contribution to the advancement of the discipline. They confirm the research independence and competencies required for a habilitation degree. A key element of the cycle is the integration of advanced biotechnological techniques with clinical and molecular analysis of both the virus and the host. An important achievement is the development of an innovative method for detecting covalently closed circular DNA (cccDNA) through selective digestion of rcDNA with T5 exonuclease, which enabled precise analysis of this viral form in clinical samples by eliminating interfering forms. As a result, it was demonstrated that cccDNA forms serve as a reservoir for drug resistance mutations, which are often undetectable in plasma. This finding is of significant importance for monitoring the effectiveness of therapy and designing strategies for HBV elimination.

In parallel, extensive analyses of host genetic variability were conducted, identifying single nucleotide polymorphisms (SNPs) in genes involved in the viral replication cycle, immune response to infection, hepatocyte metabolism, and DNA repair pathways. For both viral genome analysis and host genetic variability assessment, a high-throughput MALDI-TOF mass spectrometry method was employed, which further integrated the entire publication cycle and highlighted its methodological coherence and translational significance. Subsequently, by utilizing bioinformatic analytical and statistical tools, predictive and prognostic biomarkers (Table 5) were identified, which can support the personalization of therapy and assessment of the risk of progression to cirrhosis and HCC. As a result, it was possible to develop an integrated diagnostic approach that combines molecular virology with modern human genomics in the context of chronic HBV infection.

Table 5. Summary of markers identified in the presented series of publications according to key clinical aspects.

Marker/Gene (Variant)	Clinical Effect	Publication
Markers of Liver Disease Progression		
<i>HNF1A</i> (rs2464196)	AA genotype: advanced fibrosis and inflammatory activity	3
<i>HNF1A</i> (rs7310409)	AA genotype: increased inflammatory activity	3
<i>TDP2</i> (rs1047782)	GG genotype: increased inflammatory activity	3
<i>XRCC1</i> (rs25487)	TT genotype: cirrhosis	7
<i>ERCC2</i> (rs238406)	GG genotype: cirrhosis	7
<i>NBN</i> (rs2735383)	GG genotype: cirrhosis	7
<i>DIO2</i> (rs225014)	TT genotype: advanced fibrosis	8
<i>GADD45A</i> (rs532446)	TT genotype: cirrhosis	8
<i>PPARG</i> (rs10865710)	CC genotype: advanced fibrosis	8
<i>ATF3</i> (rs11119982)	TT genotype: cirrhosis	8
<i>TNF-α</i> (rs1799964)	TT genotype: increased inflammatory activity	4
<i>TNF-α</i> (rs1800630)	CC genotype: increased inflammatory activity	4
<i>TNF-α</i> (rs1799724)	CT genotype: increased inflammatory activity	4
<i>IL-10</i> (rs1800871)	TT genotype: less severe liver fibrosis	5
<i>IL-10</i> (rs1800872)	AA genotype: less severe liver fibrosis	5
<i>IL-10</i> (rs3024490)	TT genotype: less severe liver fibrosis	5
<i>IL-10</i> (rs1518110)	TT genotype: less severe liver fibrosis	5
cccDNA HBV	G1764A mutation: increased inflammatory activity	2
Markers of Treatment Response		
<i>NTCP</i> (rs7154439)	AA genotype: more frequent HBeAg seroconversion (NRTI)	3
<i>TDP2</i> (rs3087943)	AA genotype: more frequent HBsAg elimination (Peg IFN α 2a)	3
<i>HNF1A</i> (rs1169288)	TT genotype: more frequent HBsAg elimination (Peg IFN α 2a)	3
<i>HNF1A</i> (rs7310409)	GG genotype: more frequent HBsAg elimination (Peg IFN α 2a)	3
<i>IL-10</i> (rs1800871)	T allele: more frequent HBsAg elimination (Peg IFN α 2a)	5
<i>IL-10</i> (rs1800872)	A allele: more frequent HBsAg elimination (Peg IFN α 2a)	5
<i>IL-10</i> (rs3024490)	T allele: more frequent HBsAg elimination (Peg IFN α 2a)	5
<i>HNF4A</i> (rs1800961)	T allele: sustained treatment response	3
Markers of Viremia and Viral Replication		
<i>TNF-α</i> (rs1799964)	TT genotype: higher HBV DNA level	4
<i>TNF-α</i> (rs1800630)	CC genotype: higher HBV DNA level	4
<i>IL-10</i> (rs1800896)	AA genotype: lower HBV DNA level	5
<i>IL-10</i> (rs1800893)	CC genotype: lower HBV DNA level	5
cccDNA HBV	G1764A mutation: elevated liver enzyme levels	2
<i>AKT3</i> (rs12031994)	TT genotype: higher HBsAg level	8

Key achievements of the presented habilitation thesis proposal:

- Development of a sensitive method for analyzing the cccDNA form of the HBV genome.
- Detection and comparison of sequence differences between HBV rcDNA and cccDNA forms, as well as their significance in disease progression and therapy.
- Identification of host genetic factors influencing infection course, disease progression, and treatment response.
- Definition of the clinical relevance of SNP polymorphisms (*TDP2*, *NTCP*, *HNF1A*, *HNF4A*) in the course of HBV.
- Determination of the impact of *TNF- α* polymorphisms on infection chronicity and treatment response.
- Analysis of *IL-10* polymorphisms in the context of infection progression and antiviral therapy effectiveness.
- Investigation of the role of DNA repair gene polymorphisms (*ERCC2*, *XRCC1*, *NBN*) in the risk of cirrhosis and fibrosis progression.
- Identification of new biomarkers of disease progression risk, enabling personalized treatment and patient monitoring.

4.4 Description of other scientific achievements and accomplishments

After obtaining my doctoral degree, my scientific activity expanded significantly and included participation in numerous research projects and publications, which contributed to deepening my experience and developing my research skills. Following the completion of the international INFECT-ERA project, in which I was initially involved as an assistant before obtaining my doctorate and later as an adjunct, I continued serving as a scientific and technical staff member in the Core Facility Laboratories, with a focus on the Laboratory of Genetic Analysis. In this role, I participated in numerous research projects, supporting scientific teams in conducting genetic studies, processing results, and preparing scientific publications. My involvement included both the practical aspects of laboratory research and substantive support in experiment planning and data analysis. Beyond the publication cycle detailed in Section 4.2, my additional scientific achievements include seven original experimental papers published between 2021 and 2025, with a combined impact factor of **32.618**.

Special attention among the works outside the main cycle should be given to three papers related to the topic of **modern diagnostic and prognostic methods in infectious diseases**, particularly viral ones. These works concern innovative diagnostic approaches, including the use of molecular techniques and the identification of prognostic markers in chronic viral infections, such as hepatitis C virus (HCV) infection or SARS-CoV-2.

List of articles and their descriptions:

1. **Rybicka M**, Miłosz E, Bielawski KP. Superiority of MALDI-TOF Mass Spectrometry over Real-Time PCR for SARS-CoV-2 RNA Detection. *Viruses*. 2021 Apr 22;13(5):730. doi: 10.3390/v13050730. PMID: 33922195; PMCID: PMC8145549. IF₂₀₂₁=5,818 MEiN₂₀₂₁=100

My contribution to the publication: I played a leading role throughout the entire research process—from designing the study concept, developing the methodology and software, to supervising the project's implementation. I was responsible for data analysis, validation of results, conducting experiments, data processing, and preparing the original manuscript. I also participated in reviewing and editing the text, visualizing the results, project administration, and securing funding. I was also serving as the corresponding author.

2. Wróblewska A, Woziwodzka A, **Rybicka M**, Bielawski KP, Sikorska K. Polymorphisms Related to Iron Homeostasis Associate with Liver Disease in Chronic Hepatitis C. *Viruses*. 2023 Aug 9;15(8):1710. doi: 10.3390/v15081710. PMID: 37632052; PMCID: PMC10457817. IF₂₀₂₃=3,8 MEiN₂₀₂₃=100

My contribution to the publication: As part of the team effort, I participated in developing the research methodology, conducting experiments, and validating the results. I was also involved in editing and proofreading the manuscript.

3. Wróblewska A, Gliwiński M, **Rybicka M**, Cheba M, Lorenc B, Trzonkowski P, Bielawski KP, Sikorska K. Residual HCV-RNA and Elevated Platelet-to-Lymphocyte Ratio Predict Poor Long-Term Outcomes in Patients with Chronic Hepatitis C After Treatment. *Infect Dis Ther*. 2025 Jan;14(1):305-315. doi:10.1007/s40121-024-01101-2. Epub 2024 Dec 26. PMID: 39725828; PMCID: PMC11782785. IF₂₀₂₄=5,3 MEiN₂₀₂₄=140

My contribution to the publication: As part of the team effort, I participated in developing the research concept and methodology, preparing samples for the study, as well as collecting and analyzing data. I was also involved in editing and proofreading the manuscript.

Total publication impact factor (IF): 14.918

Total number of MEiN points: 300

The first article, "**Superiority of MALDI-TOF Mass Spectrometry over Real-Time PCR for SARS-CoV-2 RNA Detection**" (*Viruses*, 2021), presents a comparison of the diagnostic effectiveness of the MALDI-TOF mass spectrometry technique with the clinically approved real-time RT-PCR method for detecting SARS-CoV-2. The study group consisted of 168 patients suspected of infection and showing symptoms of respiratory tract infection. When the same samples were analyzed simultaneously by both methods, inconsistent results were obtained in 17 cases, accounting for 10.12% of all analyzed samples. Among the 15 samples officially reported as initially positive, 13 tested positive with the mass spectrometry-based test. Additionally, two samples considered negative by the approved RT-PCR test were positive in the mass spectrometry test. The study clearly demonstrated the higher sensitivity of the mass spectrometry-based test in detecting SARS-CoV-2, indicating that MALDI-TOF MS technology may be an ideal tool not only for virus detection but also for identifying mutations in its genome. The same technology (MALDI-TOF MS) was used in the study titled "**Polymorphisms Related to Iron Homeostasis Associate with Liver Disease in Chronic Hepatitis C**" (*Viruses*, 2023). Its application focused on the analysis of genetic polymorphisms related to iron metabolism as potential risk factors for liver damage in the course of HCV infection. The results obtained showed an association between certain genetic variants (e.g., in the *HDAC3* and *CYBRD1* genes) and the severity of histopathological changes, an increased risk of developing HCC, as well as alterations in the expression of immune receptors in the liver. These findings suggest that individual genetic susceptibility may significantly influence the course of chronic HCV infection and should be considered when assessing the risk of disease progression. Third article, "**Residual HCV-RNA and elevated platelet-to-lymphocyte ratio predict poor long-term outcomes in patients with chronic hepatitis C after treatment**" (*Infectious Diseases and Therapy*, 2025), focuses on determining the role of residual HCV RNA presence in peripheral blood mononuclear cells (PBMCs), known as occult hepatitis C infection (OCI), and

prognostic indicators in patients with chronic HCV infection after completing treatment with direct-acting antivirals (DAA). A total of 42 patients were observed, with OCI status assessed after therapy over a median follow-up of 6.3 years. It was shown that the presence of residual HCV RNA (>12 weeks post-therapy) is associated with a fivefold increased risk of liver fibrosis progression and an elevated platelet-to-lymphocyte ratio (PLR), which was strongly linked to adverse clinical outcomes, including liver disease progression and increased mortality. This work highlights the necessity for long-term monitoring of patients after HCV treatment and the search for new, non-invasive risk markers.

The presented publications form a logical sequence of research—from the development and validation of modern diagnostic tools (MALDI-TOF MS for SARS-CoV-2 detection), through the identification of prognostic markers (residual HCV RNA, PLR), to the analysis of genetic factors influencing the risk of complications in chronic viral infections (HCV). This cycle reflects current trends in laboratory and clinical medicine, where the following are of key importance: precise molecular diagnostics, personalized risk assessment, and integration of genetic and immunological data into everyday clinical practice.

My research interests are currently expanding to include **innovative treatment methods, such as the development of new anticancer drugs, biological therapies and substances with antimicrobial potential**. As part of a scientific collaboration with Prof. Marta Fiołka, PhD, DSc (Department of Immunobiology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University in Lublin), I participated in research on an innovative anticancer drug—Venetin-1, which is a nanoparticulate protein-polysaccharide complex isolated from the coelomic fluid of the earthworm. As part of the conducted research, it was demonstrated that Venetin-1 has strong anticancer activity against non-small cell lung cancer (NSCLC), both in *in vitro* and *in vivo* models (**Scientific Reports 2022**). An in-depth analysis of the molecular mechanisms of Venetin-1 action on A549 lung cancer cells enabled the identification of signaling pathways and proteins whose expression undergoes significant changes in response to Venetin-1 treatment (**Frontiers in Molecular Biosciences 2023**). The next compound analyzed was oleanolic acid—a natural compound with neuroprotective properties. The results obtained in the studies confirmed that oleanolic acid protects nerve cells from oxidative damage and apoptosis. Its efficacy was confirmed in an animal model of neurodegenerative diseases (**Pharmaceuticals 2023**). The most recent publication (**International Journal of Molecular Sciences 2025**) concerns the use of IgY antibodies obtained from chicken egg yolk in combating zoonotic pathogens such as *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. *In vitro* studies have shown that these antibodies effectively inhibit bacterial growth, confirming their potential as an alternative to antibiotics in the prevention and treatment of infections. The first author of this publication is Ms. Paulina Czoska, whose doctoral thesis I am co-supervising. Below, I present the most

important publications in this area of research, along with a detailed description of my contribution to their development.

1. **Rybicka M**, Czapplewska P, Rzymowska J, Sofińska-Chmiel W, Wójcik-Mieszawska S, Lewtak K, Węgrzyn K, Jurczak P, Szpiech A, Nowak J, Musiał N, Fiołka MJ. Novel Venetin-1 nanoparticle from earthworm coelomic fluid as a promising agent for the treatment of non-small cell lung cancer. *Sci Rep.* 2022 Nov2;12(1):18497. doi: 10.1038/s41598-022-21665-8. PMID: 36323731; PMCID: PMC9630273. IF₂₀₂₂=4,6 MEiN₂₀₂₂=140

My contribution to the publication: I participated in developing the research methodology as well as in the verification and validation of the *in vitro* results. I was involved in conducting the experimental studies, collaborating as part of the research team. My contribution also included support in the process of data analysis and interpretation. I took an active role in writing the initial version of the manuscript and prepared Figures 1, 2, 3, 5, as well as Table 1. I also participated in revising the manuscript after receiving reviewers' comments.

2. Czapplewska P, Bogucka A, Macur K, **Rybicka M**, Rychłowski M, Fiołka MJ. Proteomic response of A549 lung cancer cell line to protein-polysaccharide complex Venetin-1 isolated from earthworm coelomic fluid. *Front Mol Biosci.* 2023 Jun 8;10:1128320. doi: 10.3389/fmolb.2023.1128320. PMID: 37377864; PMCID: PMC10292018. IF₂₀₂₃=3,9 MEiN₂₀₂₃=140

My contribution to the publication: I developed the research methodology as well as the verification and validation of the *in vitro* results. I participated in conducting the experimental studies, collaborating within the research team. My contribution also included support in data analysis and interpretation. I took an active role in writing the initial version of the manuscript and prepared Figures 1, 2, and 3. I also participated in revising the manuscript after receiving reviewers' comments.

3. Stępnik K, Kukula-Koch W, Plazinski W, **Rybicka M**, Gawel K. Neuroprotective Properties of Oleanolic Acid-Computational-Driven Molecular Research Combined with In Vitro and In Vivo Experiments. *Pharmaceuticals (Basel).* 2023 Aug 31;16(9):1234. doi: 10.3390/ph16091234. PMID: 37765042; PMCID: PMC10536188. . IF₂₀₂₃=4,3; MEiN₂₀₂₃=140

My contribution to the publication: I developed the research methodology as well as the verification and validation of the *in vitro* results. I participated in conducting the experimental studies, collaborating within the research team. My contribution also included support in data analysis and interpretation. I took an active role in writing the initial version of the manuscript and prepared Figures 5 and 6. I also participated in revising the manuscript after receiving reviewers' comments.

4. Czoska P, Tarsalewska K, Ponichtera M, **Rybicka M***, Sowa-Rogozinska N, Sominka-Pierzchlewicz H, Stodolna A, Ogonowska P, Kosciuk A, Glosnicka R, Bielawski KP. Growth-Inhibitory Effect of Chicken Egg Yolk Polyclonal Antibodies (IgY) on Zoonotic Pathogens *Campylobacter jejuni*, *Salmonella* spp. and *Escherichia coli*, In Vitro. *Int J Mol Sci.* 2025 Jan 25;26(3):1040. doi: 10.3390/ijms26031040. PMID: 39940808; PMCID: PMC11816624. IF₂₀₂₅= 4,9; MEiN₂₀₂₅=140

My contribution to the publication: I participated in the analysis and interpretation of research data in collaboration with the entire team of authors. I co-authored the first draft of the manuscript, developing its structure, argumentation, and substantive content based on

the experimental results. My role also included integrating the findings with the current state of knowledge and preparing the text for further editing and internal review. I also served as the corresponding author. The first author of the paper is a doctoral student whose dissertation I am co-supervising—as part of this, I actively supported her scientific development by providing substantive oversight in the preparation of the publication.

Total publication impact factor (IF): 17.7

Total number of MEiN points: 560

4.5 Information on significant scientific or artistic activity carried out at more than one university, scientific institution, or cultural institution, in particular abroad.

In 2015, shortly before defending my PhD, I completed a 3-month internship in Professor Michael Nassal's group at the Laboratory of Molecular Biology, 2nd Department of Internal Medicine, University Medical Center Freiburg, Germany. I obtained funding for this internship from the National Science Centre, as a recipient of the ETIUDA2 scholarship (UMO-2014/12/T/NZ7/00335). During the internship, I was involved in a project aimed at identifying new human genes involved in the formation of the cccDNA form of the HBV virus. My role consisted of modifying the HBV genome in such a way that, while maintaining the virus's high replication potential enabling cccDNA formation *in vitro*, it also allowed for easy monitoring of the infection process in hepatocyte cells. Each of the HBV plasmids I modified was analyzed for its ability to replicate, generate all intermediate replicative forms, and synthesize viral proteins. **The results of the research I conducted during the internship formed the basis for creating an efficient HBV infection system, which is essential for a better understanding of the mechanisms involved in viral replication.** Working on this system allowed me to deepen my knowledge of the mechanisms involved in the formation of cccDNA, which is a key structure in the course of infection. The experience gained during the internship enabled me to expand my research to include a detailed analysis of the HBV cccDNA sequence. The collected data indicate that it is the cccDNA sequence, rather than the circulating relaxed circular HBV DNA (rcDNA), that plays a crucial role during antiviral therapy. This internship was an extremely valuable experience—it not only reinforced my research interests and allowed me to master new analytical methods, but also enabled me to establish valuable professional relationships that would prove beneficial in the years that followed. The numerous conversations I had with Prof. Nassal were particularly important, as they had a significant influence the further direction of my research on the cccDNA sequence. His support and mentoring approach played a crucial role in shaping my scientific interests and research ideas. During

my stay, I participated in many seminars related to the ongoing research, where I presented my results several times and led discussions on the obtained outcomes. The research results obtained during the internship were fundamental to the first description in the scientific literature of the differences in sequence between covalently closed circular DNA (cccDNA) and relaxed circular DNA (rcDNA) forms of the HBV genome, and their significance for pathogenesis and treatment of the infection (Publication No. 2 of the series). Selective digestion of rcDNA with T5 exonuclease enabled precise cccDNA isolation, allowing the identification of mutations present exclusively in this viral DNA form. These mutations are often undetectable in plasma and represent a reservoir of antiviral resistance, significantly impacting the clinical course of chronic HBV infection. This work would not have been possible without the knowledge and skills acquired during the internship. Although Professor Nassal is not listed as a co-author, his scientific guidance, constructive feedback and inspiring discussions were crucial to the successful completion of this study.

From 2014 to 2018, I was one of the key contributors of the Polish team in the international research project INFECT-ERA (Infect-Era/01/2014). As part of the project, I collaborated closely with Professor Thomas Baumert from INSERM in Strasbourg. This enabled me to access clinical samples collected within the French HEPATHER project (Therapeutic Option for Hepatitis B and C: a French Cohort, NCT01953458). Obtaining these samples significantly expanded and diversified the patient group analyzed in the project. With such a large study group and preliminary research results, we continued our collaboration within the HIPShot project (HBV Genetic and Proteomic Screen, project no. 7), which was coordinated by Prof. Baumert. The result of this collaboration is three publications included in the series forming the basis of my scientific achievement (Publications No. 5, 7, 8).

Since 2023, I have been an expert in genetic diagnostics of cancers at the Core Facility Organ Specific Medicine Laboratory, Department and Clinic of Oncological, Transplant, and General Surgery, Faculty of Medicine, Medical University of Gdańsk. This laboratory was established as part of the Excellence Initiative – Research University program and specializes in translational research, combining clinical expertise with access to biological samples and patient data. My involvement in this field began with three month internship, which provided the foundation for my current research activities and expertise. In collaboration with experienced surgeons—Prof. Jarosław Kobiela and Dr. Piotr Sychalski, specialists in colorectal cancer treatment—I am conducting joint research projects focused on developing and implementing modern diagnostic methods and patient monitoring for this type of cancer. So far, I have developed a quantitative method for ctDNA detection based on ALU repeats, which enables precise determination of ctDNA levels in perioperative liquid biopsy samples. This method makes it possible to identify trends in ctDNA concentration that correlate with disease progression. Currently, I am comparing the effectiveness of this proprietary method with the commercial ddPCR KRAS G12/G13 Screening Kit from Bio-Rad for further analysis and validation of

both solutions. The aim of this collaboration with the surgical team is to increase the accuracy and effectiveness of perioperative monitoring, which can significantly impact the quality of diagnostics and therapy for colorectal cancer patients. The results of this collaboration have not yet been published, as we are waiting for a clear, measurable effect of patient treatment

5. Information about achievements in teaching, organization, and the promotion of science or art.

5.1. Teaching achievements

Subjects taught within the teaching workload

Since my appointment as an Assistant Professor (2021) at the Department of Photobiology and Molecular Diagnostics, I have served as the coordinator of the compulsory course "Molecular Diagnostics," which is dedicated to first-year students of the supplementary Master's program at the Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk (IFB UG & MUG). Within this course, I deliver lectures in English and conduct practical classes (in both Polish and English). As the course coordinator, I am responsible for preparing teaching materials and continuously improving the content presented to students. Additionally, I also deliver lectures on "Fundamentals of Laboratory Diagnostics" for third-year undergraduate students in Biotechnology (IFB UG & MUG). I have also participated in developing two courses for the new "Marine Biotechnology" program (second-cycle studies, since 2023), run by the Faculty of Oceanography and Geography and IFB UG & MUG. This program is conducted entirely in English. As part of this program, I deliver selected lectures and practical classes in the course "Pathology and Molecular Diagnostics of Aquatic Organisms."

Supervision of diploma (thesis) students and staff training

Auxiliary supervisor of the doctoral dissertation entitled: "Infections caused by *Campylobacter jejuni* – studies on the interactions between bacteria, antibodies, and the immune system" – a thesis in the field of natural sciences, in the discipline of biotechnology – in progress (defense planned for the end of 2025) – MSc Paulina Czoska.

Supervisor of 7 master's theses in Biotechnology at the IFB UG & MUG

- 2020: Maria Marimon Castelltort
- 2022: Zuzanna Szafranek
- 2023: Patrycja Markiewicz, Ainhoa Gomez Garcia
- 2024: Julia Sadowska

- 2025: Rozalia Richert, Stefania Jackowska

Supervisor of 9 bachelor's theses in Biotechnology at the IFB UG & MUG

- 2022: Julia Sadowska, Ewelina Chodowiec
- 2023: Weronika Dukat, Rozalia Richert, Ewa Nowak
- 2024: Marta Rolbecka, Natalia Buć
- 2025: Szymon Niemczuk, Aleksandra Bondyra

Reviewer of the master's thesis by MSc Martyna Muszyńska (2023)

5.2. Organizational achievement

- Participation in organizing workshops for people from the University of Gdańsk as well as external participants: 29-31.10.2013 and 14-16.01.2014
- Organization and delivery of mass spectrometry workshops for participants from the University of Gdańsk as well as external participants: 27.03.2014
- Organization and delivery of training for students of the Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk: 22-23.04.2023 - Applications of Mass Spectrometry in DNA Analysis

5.3. Achievements Popularizing Science

- Participation in organizing scientific activities for students of class 6B from the II Social Primary School STO in Gdańsk.

6. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

6.1 Participation in Research Projects and Grants

Participation in National Research Projects

- **2014-2017 PRELUDIUM 6** (leader and executor) – source of funding: National Science Centre (2013/11/N/NZ7/00377). As part of the project, I was responsible for collecting research material, designing and conducting experiments, as well as analyzing and interpreting the obtained results. I prepared reports for the National Science Centre, drafted manuscripts, and presented research findings at national and international conferences.

- **2018-2020 OPUS** (executor) – source of funding: National Science Centre (2016/22/E/HS6/00237). In this project, I was responsible for all genetic analyses. I planned experiments, developed and optimized research procedures, analyzed data, and wrote manuscripts.

Participation in international projects:

- **2013-2016 MOBI4HEALTH** (executor) – source of funding: European Commission, 7th EU Framework Programme under the REGPOT call (FP7-REGPOT-2012-2013-1). As part of the MOBI4Health project, I actively participated in the organization and implementation of scientific events, such as conferences and workshops, which fostered the integration of the Faculty with the European Research Area. I completed numerous specialized courses in mass spectrometry techniques, which enabled me to carry out advanced research projects. I shared the experience I gained by conducting workshops for both university staff and individuals from outside the University of Gdańsk. Additionally, I actively promoted the modern research infrastructure of the Faculty of Biotechnology at numerous national and international conferences.
- **2014-2018 INFECT-ERA** (executor)– source of funding: European Commission, 7th EU Framework Programme under the Infect-ERA call (Infect-Era/01/2014). My role included planning, optimizing, and conducting experiments, supervising the collection of research material in Poland, creating and managing a database, as well as performing statistical analyses. I was responsible for preparing and submitting applications to the French bioethics committee and coordinating the transfer of samples from France to Poland. I regularly represented the Polish team at project meetings—both during videoconferences and in-person meetings in Strasbourg—where I presented the results obtained by our research group at an international forum. Participation in these meetings enabled me to actively exchange experiences and ideas with international partners, which significantly enriched our scientific collaboration and contributed to achieving the project's objectives. The results obtained during the implementation of the INFECT-ERA project formed the basis for preparing several original publications and one review paper, and also laid the foundation for further scientific cooperation within subsequent international projects, including HIPShot, coordinated by Prof. Baumert.

3.1. Awards and Distinctions

Awards and scholarships

- Annual scholarship from the so-called "pro-quality subsidy", awarded to a group of the best doctoral students doing doctoral projects at IFB-UG and MUG (years 2011/2012, 2013/2014, and 2014/2015)
- Annual scholarship for the best PhD students at IFB-UG and MUG (years 2011/2012, 2013/2014, and 2014/2015)
- Annual scholarship as part of the project "We educate the best - a comprehensive program for the development of doctoral students, young doctors and academic teaching staff of the University of Gdańsk". Awarded in a competition. Project financed by the European Social Fund under the Human Capital Operational Program (2011/2012)
- Pomeranian Special Economic Zone (PSSE) scholarship, awarded in a competition based on applications submitted under the 5th edition of the InnoDoktorant program (2012/2013)
- Annual ETIUDA2 scholarship (2014/12/T/NZ7/00335) awarded by the National Science Center (2014/2015)
- International scholarship: EASL Award for Young Investigators – bursary awarded by the European Association for the Study of the Liver for participation in the EASL Basic School of Hepatology: Molecular Biology and Pathogenesis of Hepatitis Viruses, Switzerland, 5–7 February 2015
- Team Rector's First Degree Award of the University of Gdańsk, 2021, 2023, 2024
- Team Rector's Second Degree Award of the University of Gdańsk, 2022, 2025

Conference honors

- Distinction for a scientific poster, Falk Symposium 195: *Challenges and Management of Liver Cirrhosis*, Freiburg, Germany, 10–11.10.2014.
- Distinction for a scientific poster, Falk Symposium *New Treatment Targets in Gut and Liver Disease*, Lucerne, Switzerland, 21–22.10.2016

6.4 Reviewing Scientific Manuscripts:

So far, I have reviewed 30 scientific manuscripts submitted for publication in international journals such as Scientific Reports, International Journal of Biological Sciences, Clinical and Experimental Gastroenterology, Journal of Hepatocellular Carcinoma, OncoTargets and Therapy, Discover Oncology, BMC Infectious Diseases, and others.

7. Bibliometric analysis of publications presented in the application

Works constituting the basis for the habilitation IF: 34.433

Works constituting other scientific achievements IF= 32.618

Total IF achievements 67.051

Total MEiN Score: 1552

Hirsch index: 8

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(Applicant's signature)

8. Bibliography

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