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INTERRELATIONSHIP BETWEEN CLINICAL PHENOTYPE AND GENOTYPE IN PATIENTS WITH WILSON'S DISEASE

321.01 – INTERNAL DISEASES (GASTROENTEROLOGY AND HEPATOLOGY)

Summary of the doctoral thesis in medical sciences

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CONTENT

INTRODUCTION

Current status and importance of the problem addressed. Wilson's disease (WD), also known as lenticular degeneration, is an autosomal recessive pathology caused by mutations in the ATP7B gene, located on chromosome 13 (gene locus: 13q 14.3–921.1) [1]. Dysfunction of the ATP7B protein results in impaired biliary excretion of copper (Cu) and, consequently, in the accumulation of Cu in the liver and extrahepatic tissues. The clinical picture is very varied, with hepatic and neurological involvement being the main forms of presentation. The disease can become symptomatic at any age or may remain asymptomatic for an indefinite period. [2]

Over 900 pathogenic variants of this gene have been identified, and efforts to associate them with disease phenotypes have been inconclusive and controversial [3]. The difficulty in establishing genotype-phenotype correlation can be attributed to several factors: the large number of mutations that occur in only a few families and the clinical heterogeneity of WD patients, even among members of the same family. The fact that most patients are compound heterozygotes, having different mutations on each allele, makes it difficult to correlate a phenotype with a mutant allele [4]. In addition, occupational exposure to Cu has been shown to cause genome-wide changes and DNA damage [5]. This aspect, in combination with epigenetic modulators and environmental factors, may play a role in the phenotypic heterogeneity of WD patients. These barriers can be partially overcome by studying the genetics of WD in homozygous patients. [6] A multifamily study in an isolated population with a high degree of genetic and environmental homogeneity from a mountainous region of Romania revealed an equal influence of putative genetic modifiers and environmental factors on the clinical presentation and age at onset of WD in patients with a given genotype [7].

Specific mutations in the ATP7B gene are more common in populations with consanguineous marriages. For example, in Lebanon, consanguinity has a prevalence of 35.5%, increasing the likelihood of homozygosity for autosomal recessive diseases. [8] Chabik and coauthors have highlighted the presence of a similar clinical and biochemical manifestation of WD with a high intra-familial concordance, suggesting that analogous factors shared in the same families may strongly influence the presentation of the disease [4]. On the other hand, some authors have shown significant differences in phenotype despite the same genotype, suggesting a significant influence on the WD phenotype by epigenetic/environmental factors [9, 10]. A study from China has demonstrated a relationship between some pathogenetic variants, different clinical forms, and the occurrence of WD. For example, the *p.Arg919Gly* mutation was associated with neurological disease, and *p.Arg778Leu* was associated with a younger age of onset and lower serum ceruloplasmin (Cp) and Cu levels. However, the study only included the Chinese population, so further studies are needed in Caucasian patients with WD. [11] Furthermore, the incidence of this disease in certain regions seems to be closely related to the so-called “founder effect,” indicating the property of certain “isolated” communities to contract autosomal recessive genetic diseases more frequently, because mutations that are normally rare are found much more frequently in these communities [12].

However, recent research has established that the heterogeneity of clinical phenotypes does not only depend on the degree of functional alteration of ATP7B or genetic mutation but also on pathogenic mutations in other genes involved in Cu homeostasis, defined in the same studies as *“copper homeostasis genes”* or CHGs [13], which could be analyzed in the future. Future research may change the therapeutic approach of WD patients about their genotype, suggesting the need for

combination therapy in case the most severe pathogenic mutations, associated with ATP7B and/or CHG polymorphisms, are present.

Currently, a complex approach is required in WD management, analyzing the genotype-phenotype correlation in the light of identifying diagnostic/screening, treatment, and prognosis possibilities.

Research hypothesis. As a result of this research for the first time in the Republic of Moldova, we will be able to identify a genetic profile of the patient with WD, which will allow the identification of mutations involved in the development of the disease on the territory of the country, as well as the inherited status (homozygous recessive, compound heterozygous, simple heterozygous and without mutations detected at the level of the ATP7B gene). Belonging to a clinical phenotype will allow the determination of the predominant clinical presentation as well as factors that could influence the clinical evolution of the disease. Highlighting associations between phenotype and genotype would highlight new solutions in the diagnosis and treatment of the patient with WD. Familial evaluation of the proband would identify new family members in an asymptomatic clinical stage, which would allow the initiation of early therapy with the prevention of complications and irreversible lesions.

The purpose of the scientific research is to study and establish the correlation between the clinical phenotype and genotype of the patient with Wilson's disease in order to develop an algorithm for diagnosis and family screening of patients with Wilson's disease.

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To achieve the goal, the following **general research objectives** were proposed:

1. Identifying demographic aspects in patients with Wilson's disease in the Republic of Moldova;
2. Defining clinical phenotypes determined in patients with Wilson's disease;
3. Characterization of the spectrum of genetic mutations in patients with Wilson's disease;
4. Assessing clinical-genetic interrelationships in patients with Wilson's disease;
5. Studying the impact of family screening in patients with Wilson's disease;
6. Developing a diagnostic and familial screening algorithm for patients with Wilson's disease.

General methodology of scientific research. In order to achieve the purpose and objectives of the research, an observational analytical cohort study was planned, with a bidirectional character. The study included the entire volume of patients suspected or diagnosed with WD. Patients were selected from the hepatology department of the Public Medical and Sanitary Institute (PMSI) Republican Clinical Hospital "Timofei Mosneaga", which is the clinical basis of the Gastroenterology Discipline of the State University of Medicine and Pharmacy (SUMP) "Nicolae Testemitanu", as well as from the Human Molecular Genetics Laboratory, part of the Genetic Center of Excellence of the Republic of Moldova, within the PMSI Institute of Mother and Child. According to the research protocol, three stages of patient evaluation were performed: the pre-screening stage, the screening stage, and the phenotyping stage. Confirmation of the suspected diagnosis at the pre-screening stage, where patients were evaluated clinically, biochemically, and imaging, was established by the modified Leipzig score, which included genetic evaluation. The data obtained in the study were statistically analyzed, and its results served as the basis for formulating conclusions.

The research was conducted based on the positive opinion of the Research Ethics Committee (verbal process no. 1 of May 25, 2021) and based on the decision of the Scientific Council of the Consortium no. 1/4.16 of 02.12.2021 regarding the approval of the research project, topic, scientific supervisor, co-supervisor and the supervision committee for the doctoral thesis in medical sciences.

The novelty and originality of the study. For the first time in the Republic of Moldova, a complex study was conducted that analyzes not only the clinical profile of the patient with WD, but also the interrelation between genotype and phenotype in patients with WD, which makes an important contribution to the field of medical genetics and hepatology. The research introduces an integrated model for analyzing patients with WD taking into account demographic variables related to age and sex.

For the first time, all variants identified in patients with WD were listed and defined according to genetic and phenotype parameters. They were also distributed by regions (north, center, south and the left Nistru region), thus highlighting the association of mutations with certain territorial regions in the country.

Aspects related to the onset of the disease and the duration of diagnosis of WD, as well as its impact on the possibilities of diagnosis, treatment and prognosis, were highlighted. Comparative studies between different groups of patients differentiated by criteria related to the type of inherited mutation have highlighted the interaction between the mutation and environmental factors, as well as their influence on the manifestation of the disease.

For the first time, an algorithm for multidisciplinary clinical evaluation of the suspected patient with WD was proposed, as well as an algorithm for evaluating family members of patients suffering from this disorder. These aim to facilitate the management of the suspect person and the steps necessary to finalize the diagnosis, as well as the subsequent tactics of conduct depending on the score obtained.

Theoretical significance and applicative value of the work. The implementation of the retrospective observational study contributed to filling in some gaps in the current scientific evidence in the field, noted in meta-analyses and international systematic reviews. The results obtained have a direct applicability in medical practice and clinical genetics with the aim of improving the diagnosis, treatment and management of WD. The analysis of the group according to the predominantly affected organ highlights the characteristics of each phenotype, essential in establishing the diagnosis and monitoring the patient, and the illustration of concrete case studies demonstrates the direct role of the results on clinical practice. Delay in diagnosis is one of the factors that cause liver decompensation and irreversible neurological damage despite the subsequent initiation of specific therapy, and the development of strategies at the state policy level would increase medical vigilance and awareness of the general population. The proposed algorithm for diagnosis and screening can be used in genetics and gastroenterology centers, and the practical recommendations provided contribute to optimizing patient management. The study highlights the role of family screening for detecting individuals at risk within the families of patients known to have WD, offering the possibility of proactive monitoring and prevention of severe complications. Thus, a subgroup of asymptomatic patients, carriers of pathogenic variants, who initiated early treatment with chelators were identified. This highlights the economic impact of early diagnosis by reducing costs related to preventing irreversible

complications, and the use of these results in public health policies would have significant benefits on the population and the medical system.

Approval of the research results. The study results and the clinical aspects investigated were reflected in discussions at national and international forums (Moldova, Romania, Spain, Japan, Netherlands), as well as at the annual conferences of collaborators and students of the "Nicolae Testemitanu" SUMF:

- EASL Congress 2025 (Amsterdam, Netherlands, 2025);
- National Conference “Rare Disease Day 2025” (Chisinau, Republic of Moldova, 2025);
- The 5th UpDate on Hepatology Course: Refocusare și Resetare în Boli Hepatice (Chisinau, Republic of Moldova, 2024);
- The 43rd National Congress of Gastroenterology, Hepatology, and Digestive Endoscopy (Cluj Napoca, Romania, 2024);
- The 10th International Medical Congress for Students and Young Doctors MedEspera (Chisinau, Republic of Moldova, 2024);
- The 33rd Annual Meeting of the Asian Pacific Association for the Study of the Liver (APASL) (Kyoto, Japan, 2024);
- The 4th UpDate on Hepatology Course: Rare Liver Diseases – Transition of Medical Care from Child to Adult (Chisinau, Republic of Moldova, 2023);
- 1st ERN RARE-LIVER EASL ACADEMY (Barcelona, Spain, 2023);
- Annual Scientific Conference - Research in Biomedicine and Health: Quality, Excellence and Performance. USMF “Nicolae Testemitanu” (Chisinau, Republic of Moldova, 2023);
- The 37th Balkan Medical Week ”THE PERSPECTIVES OF BALKAN MEDICINE IN THE POST COVID-19 ERA” (Chisinau, Republic of Moldova, 2023);
- The 42nd National Congress of Gastroenterology, Hepatology, and Digestive Endoscopy (Iasi, Romania, 2023);
- The 10th UpDate on Hepatology Course (Bucharest, Romania, 2023);
- The 3rd UpDate on Hepatology Course: New Frontiers in Hepatology (Chisinau, Republic of Moldova, 2022);
- Annual Scientific Conference - Research in Biomedicine and Health: Quality, Excellence and Performance. USMF “Nicolae Testemitanu” (Chisinau, Republic of Moldova, 2022);
- The 2nd UpDate on Hepatology Course: Precision Hepatology; December 17 - 18, 2021, (Chisinau, Republic of Moldova);
- Annual Scientific Conference Dedicated to the 76th Anniversary of the Foundation of the “Nicolae Testemitanu” SUMP (Chisinau, Republic of Moldova, 2021).

The results obtained were approved at the meeting of the Gastroenterology Discipline, Department of Internal Medicine (verbal process no. 02 of November 12, 2024) and at the meeting of the profile Seminar 321. General Medicine / Specialty: 321.01. Internal Diseases (Gastroenterology, Hepatology); 321.02. Endocrinology; 321.08 Dermatology and Venereology (verbal process no. 01 of January 20, 2025).

Publications on the research topic. The thesis materials were reflected in 20 publications, of which: 5 - were articles in journals from the National Register of Specialized Journals, 11 - were materials/theses at international conferences, and 4 - were materials/theses at national conferences. Based on the results obtained in the research conducted, 2 innovation certificates and 2 implementation acts were registered.

Summary of the thesis sections. The thesis is written in Romanian as a manuscript. The work is presented on 141 pages of text and includes: list of abbreviations, tables and figures, introduction, 5 chapters, general conclusions, practical recommendations, list of bibliographical references with 176 sources, annexes, declaration of responsibility, list of publications/participations in scientific forums and author's CV. The work contains 7 tables, 13 figures and 30 annexes.

Keywords: Wilson's disease, ATP7B gene, pathogenic mutation, genotype-phenotype correlation, copper parameters, family screening, genetic testing.

THESIS CONTENT

1. WILSON'S DISEASE – ASSESSMENT OF GENOTYPE – PHENOTYPE INTERRELATIONSHIP THROUGH THE PRISM OF THE CLINICAL–EVOLUTIONARY PROFILE

This chapter reflects information related to epidemiology, contemporary views on the etiopathogenesis of WD, genetic variability, phenotypic diversity and classification of clinical presentation, difficulties in phenotype-genotype correlations and the involvement of epigenetic mechanisms, diagnostic challenges and the role of the Leipzig score in establishing the diagnosis, as well as the impact of family screening in WD.

2. MATERIALS AND METHODS

2.1.General characteristics of the research

The patient sample is not calculated because WD is a rare genetic disease. Thus, the study included the entire volume of patients suspected or diagnosed with this disease. The patients were selected from the hepatology department of the PMSI Republican Clinical Hospital "Timofei Mosneaga", which is part of the Gastroenterology Discipline of the SUMP "Nicolae Testemitanu", as well as from the Human Molecular Genetics Laboratory, part of the Genetic Center of Excellence of the Republic of Moldova, within the PMSI Institute of Mother and Child.

The study group was defined according to the inclusion and exclusion criteria:

Inclusion criteria:

1. Modified Leipzig score ≥ 4 points;
2. Patient aged ≥ 5 year;
3. Consent of the patient or legal representative to participate in the study.

Exclusion criteria:

1. Modified Leipzig score < 4 points;
2. Patient < 5 years of age;
3. Presence of other acute and chronic liver diseases;
4. Lack of consent of the patient or legal representative to participate in the study.

Thus, an observational analytical cohort study was planned to achieve the goal and objectives. The research included several stages and took place in the period 2020 – 2024. The study design is represented in figure 1.

Stage I. Identification of the scientific concept and hypothesis - includes the study of bibliographic resources with the creation of the research concept. The problem that needs to be studied was defined and included a directed analysis of epidemiological, clinical, paraclinical, and genotype-phenotype correlation aspects, both at the global and national levels. The involvement of other factors in phenotypic diversity, as well as international practices regarding active screening in families with members affected by WD, were also investigated.

Stage II. Retrospective and prospective descriptive study of patients with WS- to define the final research group, this stage was divided into two sub-stages:

a) Prescreening – includes the entire volume of patients suspected of having WD, who presented suspicious clinical manifestations and/or Cu metabolism disorders. Also, genetic testing was performed here to identify WD-causing mutations and differentiate asymptomatic members from simple heterozygotes.
b) Screening – finalizing the research group according to the inclusion and exclusion criteria, which involves calculating the modified Leipzig score according to clinical, hematological, biochemical, imaging, and genetic parameters.

Stage III – evaluation of the research group through the prism of clinical presentation and inherited variants. Identification of genotype-phenotype interrelationships.

a) Phenotyping – distribution of a-/presymptomatic and clinically-biologically symptomatic patients according to the affected organ/system: hepatic, neuro-psychiatric and neuro-hepatic (also called mixed).
b) Genotyping – characterization of genetic mutations determined in patients according to allele frequency, variant type, inheritance status (homozygous recessive, compound heterozygous) and regional distribution.
c) Evaluation of clinical-genetic interrelationships – distribution of patients in mutational profiles according to the inherited allele (*p.H1069Q* versus non-*p.H1069Q*) and the type of variants (MMs/MMs versus MS/other-M), to assess associations between genetic expression and the phenotypic picture.

Stage IV. Processing and mathematical-statistical analysis of the material.

Ethical aspects. The individuals included in the research received the information form and signed the acceptance form for this study. Patients were assured of confidentiality during this research, all data collected from patients was stored in a database with restricted access. The approval for this research was obtained within the Research Ethics Committee of the Public Institution "Nicolae Testemitanu" SUMP – verbal process no. 1 of May 25, 2021.

2.2. Research methods applied in the study

The data were collected according to the primary assessment form of the patient with WD, developed within the research, which involved collecting the following information: personal data,

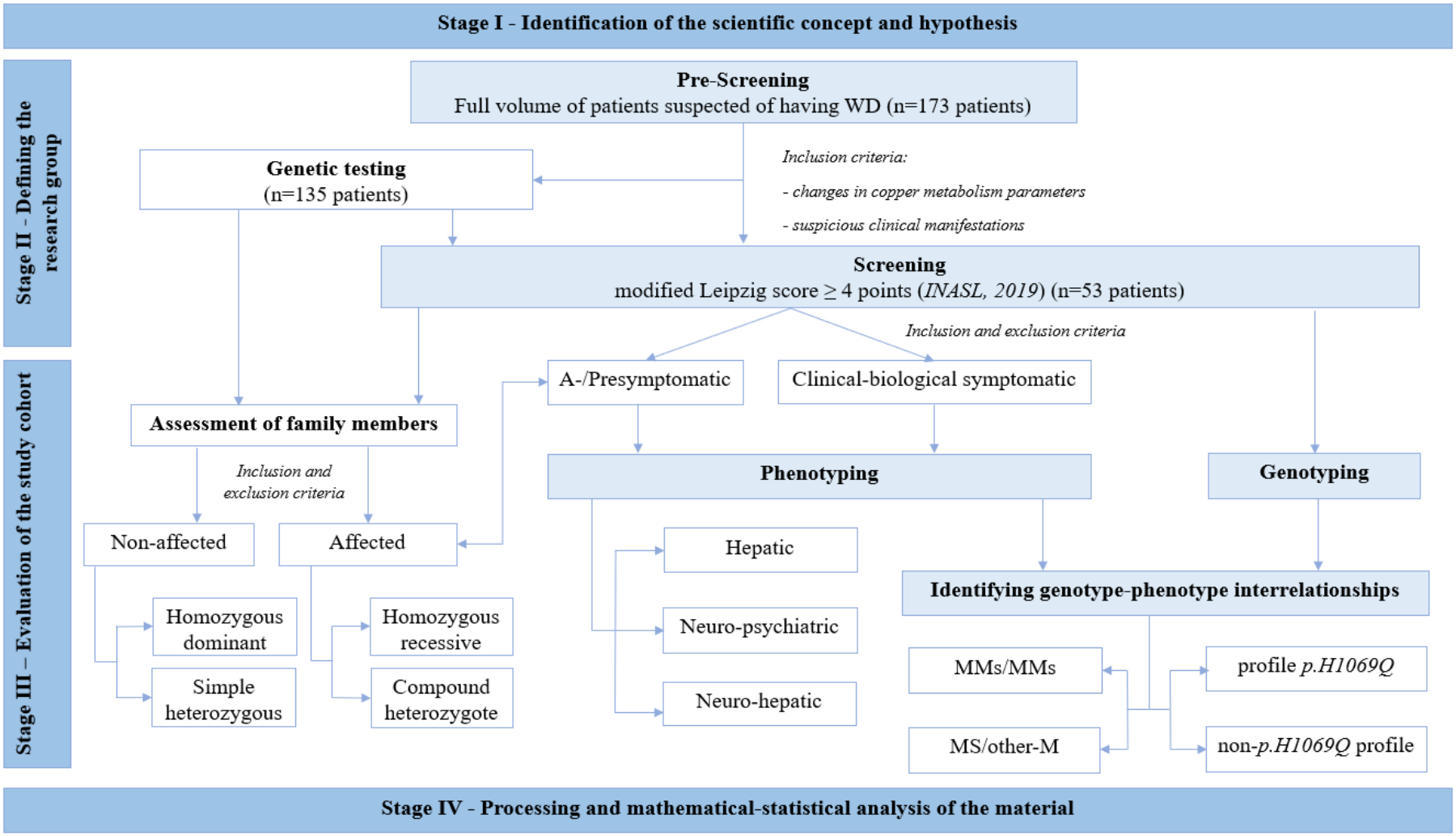

Figure 1. **Study design**

Notes: INASL - Indian National Association for Study of the Liver, MS – severe mutation; MMs – missense mutation; n – number; other-M – other mutation; WD – Wilson's disease.

demographic data, social data, social status, education and the presence of an activity. The clinical assessment included the identification of charges with the inclusion in a clinical phenotype, the presence of liver symptoms, as well as extrahepatic manifestations, signs of liver decompensation, disease history, physiological data, concomitant diseases, hereditary-collateral anamnesis, identification of vices; contact with chemical or toxic substances; allergological anamnesis; as well as physical examination. The analysis of the investigation results includes general and WD-specific laboratory tests, as well as instrumental investigations. The assessment of the degree of fibrosis was performed by applying non-invasive scores (APRI score, FIB-4). All investigations were performed both within the Family Physician Centers and at the Republican Clinical Hospital "Timofei Mosneaga" and the Republican Diagnostic Center. The involvement of the geneticist is essential in establishing the diagnosis and performing the genetic test.

Also, family members (parents, brothers/sisters, children) of patients with WD were clinically, biochemically, genetically and instrumentally evaluated, with the aim of early diagnosis of asymptomatic members and differentiating them from healthy carriers. Thus, newly identified patients initiated specific therapy until the development of symptoms.

2.2.1. Genetic testing and methods used

Molecular genetic analysis is a complex diagnostic test included in the standard of patient evaluation with WD. The genetic testing methods used in the Laboratory of Human Molecular Genetics, part of the Genetic Center of Excellence in the Republic of Moldova, are Sanger sequencing and PCR-SSCP. Molecular genetic analysis is a complex diagnostic test included in the standard of patient evaluation with WD. The genetic testing methods used in the Laboratory of Human Molecular Genetics, part of the Genetic Center of Excellence in the Republic of Moldova, are Sanger sequencing and PCR-SSCP. Sequencing was performed using Taq DyeDeoxy Terminator Cycle fluorescent dye sequencing kits (Applied Biosystems) by an ABI-Genetic 3500dx automated genetic analyzer (Applied Biosystems). The sequencing data were analyzed with the SecScape 3 software using NG_008806.1 as reference and the annotation of the identified genetic variants was based on NM_000053.3. Genetic testing from the Human Molecular Genetics Laboratory was applied by the author in the work, as a member of the team together with the geneticist.

Thus, genetic testing includes the examination of the 21 exons according to a pre-established algorithm (figure 2), depending on the frequency of mutations in the local population. In the absence of confirmation of the diagnosis, other genetic methods are recommended, such as MLPA, NGS or WES/WGS, the latter 2 being unavailable in the country. Genetic testing is indicated for confirmation of the diagnosis, family screening and in uncertain cases when the suspicion of WD persists.

2.3. Mathematical and statistical processing and analysis of the material

For the statistical evaluation of the material, the data collected from patients were entered into a database for statistical processing and subsequent digital analysis in accordance with unanimously accepted principles. The primary research materials were analyzed using the functions and modules of Microsoft Office Excel 2023 and online statistical calculators from the battery of tests offered by MedCalc https://www.medcalc.org/calc/index.ph .

For statistical processing, a set of mathematical operations performed by specific working procedures and techniques was used. Types of variables used: nominal variables, quantitative variables and interval variables. The applied statistical indicators are: arithmetic mean, standard

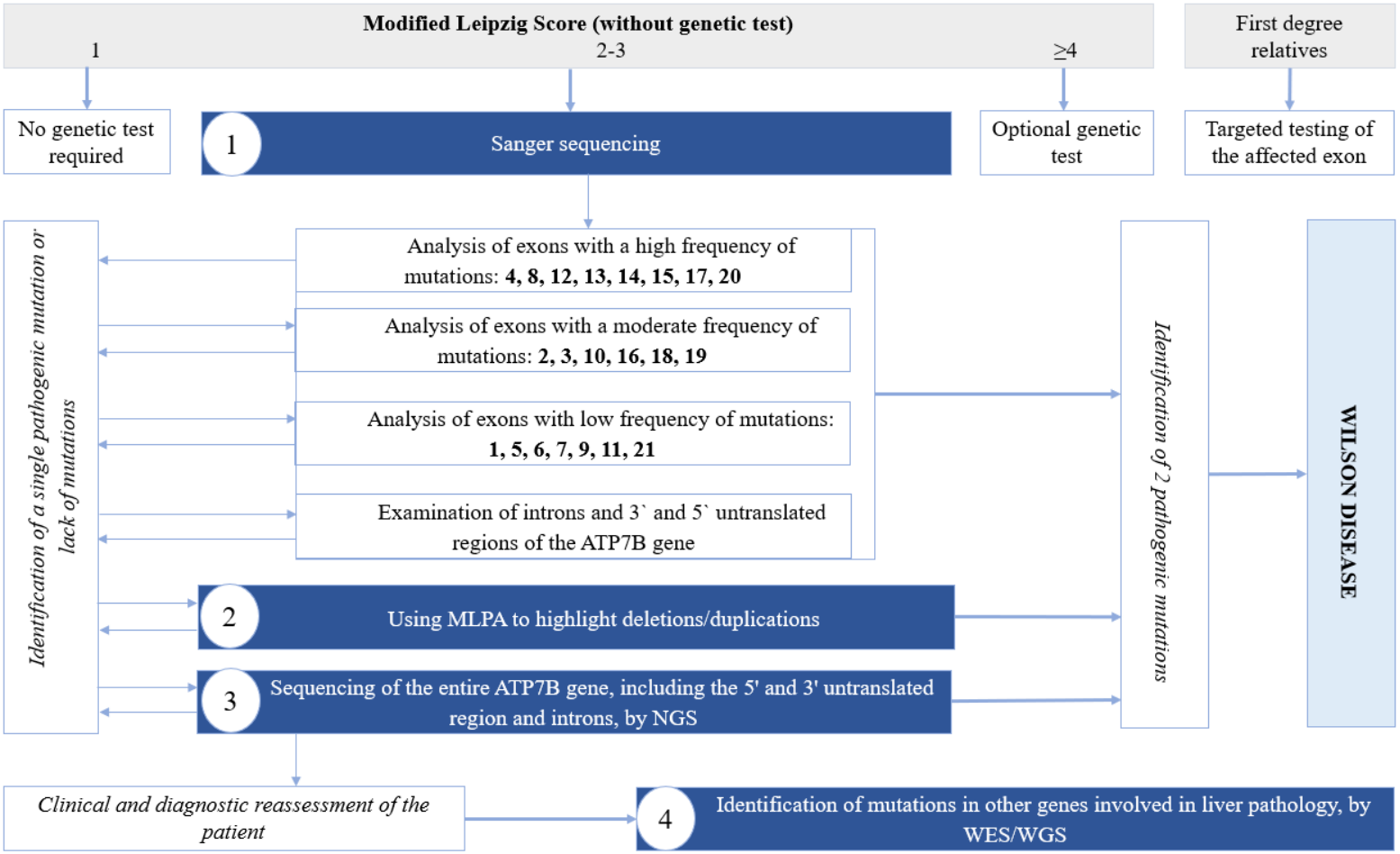

Figure 2. **Evaluation algorithm through the molecular-genetic test**

Notes: MLPA - multiplex ligation-dependent probe amplification; NGS - Next Generation Sequencing; WES/WGS - whole-exome sequencing/whole-genome sequencing - WES/WGS. *Adapted from*: Espinos C., Ferenci P. *Journal of Hepatology*. 2020 [14].

deviation (SD), median, proportion expressed in percentages of the total number of related observations and the 95% confidence interval (CI) for proportions used for nominal variables.

The statistical tests applied are: t-test, ANOVA (Analysis of Variance), Box-plot and Chi-square test. The statistical significance threshold applied to the p-value of the calculated statistics is <0.05. The presentation of statistical data was carried out through tables and graphs.

3. RESEARCH RESULTS

3.1. Identification of demographic aspects in patients with Wilson's disease

3.1.1. Identification of the study cohort by evaluating the pre-screening and screening stage

The pre-screening phase was implemented in this study, with the aim of identifying potential study participants. Pre-screening is defined as screening activity that occurs before obtaining patient consent. The pre-screening activity included reviewing lists of inpatients or outpatients suspected of having WD, reviewing patients' medical records, and preliminary discussions with potential study participants. As a result of this activity, we were able to identify not only the 53 patients with WD, but also created a cohort of patients with Cu metabolism disorders who did not meet the criteria for WD.

The non-WD group included patients with Cu metabolism disorders and/or clinical picture suggestive of WD. The Cu metabolism disorder included reduced serum Cu with/without increased urinary Cu, with/without reduced ceruloplasmin (Cp), as well as the change in free Cu (calculated according to the formula: fCu (μg/dL) = Cu (μg/dL) –3 × Cp (mg/dL) or fCu (μmol/L) = Cu (μmol/L) – 53 × Cp (g/L)). The suggestive clinical picture included non-viral hepatopathies with/without cytolysis syndrome or/and cholestasis associated with/without neuropsychiatric changes not classified as certain neurological pathologies.

Thus, of the 173 suspected patients, 120 accumulated a score for a modified Leipzig score < 4 points, and 53 obtained a score ≥ 4 points. The majority patients are young (average age 25,68 ± 16,10 years) and 73,9% cases come from the south and center of the country. Only 1/3 of them present clinical and biochemical manifestations suggestive of WD, but practically 2/3 do not meet at this stage all the criteria to establish a definite WD. Thus, applying the recommendations of the international specialist guidelines on establishing the diagnosis of WD, these patients were subjected to genetic testing. As a result, 53 patients with a definite WD were identified. This cohort was subsequently analyzed.

3.1.2. Demographic characteristics of patients with WD in the study cohort

The studied group consisted of 53 patients with WD who met the eligibility criteria. The demographic characteristics are represented in table 1. The age range varies between 5 – 46 years, with an age of <18 years there are 17 patients (32,1%). Thus, the predominance of the male sex compared to the female is observed, the male:female ratio being 1.5.

All patients are originally from the Republic of Moldova (figure 3). In the case of a multiethnic family, one of the parents is of different origin. No patient described consanguineous relationships in the family nor was he associated with any isolated community. 4 subjects do not know their family history precisely, because they grew up in an orphanage (1 case) or one of the parents was adopted

(3 cases). 16 patients report anamnestic aggravated hereditary-collateral, first and second degree relatives with confirmed WD or CLD/unspecified neurological disease.

Table 1. **Demographic characteristics of the research group**

Total patients, n=53	**Obtained value**
Male:female ratio, n (%)	32 (60,4%) : 21 (39,6 %)
Mean ± SD, years	22,62 ± 9,63
Median (25^{th} – 75^{th} percentile), years	23 (15-30)

According to the age distribution (table 2), differences are observed between the onset of the disease and the age when the patient was diagnosed, especially in the age category 5-9 years and 15-19 years. This fact highlights the lack of early establishment of the diagnosis of WD and the need for new strategies to increase the awareness of this disease. It is also attested that the maximum diagnosis of WD occurs in the period 25-29 years. The identification of WD in patients >35 years, denotes a delay in diagnosis due to a late onset with non-specific manifestations.

Table 2. **Age distribution according to onset and diagnosis**

Age, years	**Onset of the disease**		**Establishing the diagnosis**	
	n (%)	IÎ (95%)	n (%)	IÎ (95%)
5-9	9 (16,9%)	7,5 - 26,5	6 (11,3 %)	3,3 - 19,3
10-14	5 (9,4%)	2,0 - 16,8	6 (11,3 %)	3,3 - 19,3
15-19	11 (20,7%)	10,5 - 31,0	7 (13,2 %)	4,6 - 21,8
20-24	8 (15,1%)	6,0 - 24,2	9 (16,9 %)	7,5 - 26,5
25-29	11 (20,7%)	10,5 - 31,0	12 (22,6 %)	12,1 - 33,2
30-34	4 (7,5%)	0,9 - 14,2	8 (15,1 %)	6,0 - 24,2
≥35	5 (9,4%)	2,0 - 16,8	5 (9,4%)	2,0 - 16,8
Total	53 (100%)		53 (100%)	

The average duration of the diagnostic period was 26,8 ± 35,77 months, the diagnostic interval varying between 1 – 96 months. Delay in establishing the diagnosis causes liver decompensation and irreversible neurological damage, despite the initiation of specific therapy.

The analysis of patients according to the region of the country revealed that most were born/live in the Center and South, 41,5% and 32% respectively; in the North and Left Nistru, 13,2% are reported each.

By comparatively evaluating the group of patients with WD and non-WD (table 3), it was observed that although there is a visible difference in the average values in the age category, being lower in the WD group, no statistical difference was determined ($t=1,87$; $p=0.063$). In the clinical characteristic category, a statistically significant difference ($p<0,05$) is determined at all levels,

especially in the WD group where the presence of clinical and paraclinical manifestations suggestive of this disorder is observed in 86,8%, those with only paraclinical changes are asymptomatic family members detected through family screening. In the non-WD group, of the 32 patients in whom mutations were detected, the pathogenic variant on a single allele is present in more than half of the cases (56,25%), the same number being recorded in the case of benign variants. While in the WD group, of the 46 patients in whom mutations were detected, pathogenic variants are present in 93% (monoallelic – 2 cases), and benign variants in 13%. These differences between the non-WD and WD groups are statistically significant ($p<0,05$).

Table 3. **Comparative characteristics of patients in the non-WD and WD groups**

	Non-WD	**WD**
Patients, n	120	53
Mean age ±SD, years	27,1±16,2	22,62 ± 9,63
	t=1,87; p=0.063	
Median (25^{th} – 75^{th} percentile), years	25 (13-35)	23 (15-30)
Clinical characteristics, n (%)		
Clinical manifestations only	62 (51,66%)*	0 (0,0%)*
Paraclinical changes only	47 (39,17%)*	7 (13,2 %)*
Clinical and paraclinical manifestations	11 (9,17%)*	46 (86,8%)*
	χ^2=49,67, gl=2, p<0,001	
Genetic testing, n (%)	86 (71,60%)*	49 (92,45%)*
Mutations detected	32 (36,78%)*	46 (86,79%)*
Types of variants identified, n (%)	32 (100%)	46 (100%)
Pathogenic variants	18 (56,25%)*	43 (93,48%)*
Potentially pathogenic variants	0 (0,00%)	2 (4,3%)
Variants of uncertain significance	4 (4,6%)	0 (0,00%)
Benign variants	18 (56,25%)*	6 (13,04%)*
	χ^2=22,67, gl=3, p<0.001	
Evaluate family screening, n=24 (100%)	16 (66,67%)	8 (33,33%)

* statistical significance threshold between 2 compared groups: $p<0.05$

The conclusion of this subchapter is that not all patients suspected of WD, who present suggestive clinical manifestations and paraclinical changes, are established with a final diagnosis of WD. At the same time, it is necessary to highlight the fact that before establishing WD, the multitude of diseases that can mimic WD clinical symptoms must be excluded.

Thus, in our research, we followed the recommendations of international guidelines to identify patients who meet all the conditions for WD, especially those confirmed by genetic testing. As we observe, most suspects have gone through the genetic testing stage. In the study, the histological

examination of the liver biopsy was not used as a method of confirming the disease, given the unavailability of this investigation and its non-specificity. The modified Leipzig score (2019) was used as a confirmation criterion. It is a non-invasive method, with precision in establishing the final diagnosis.

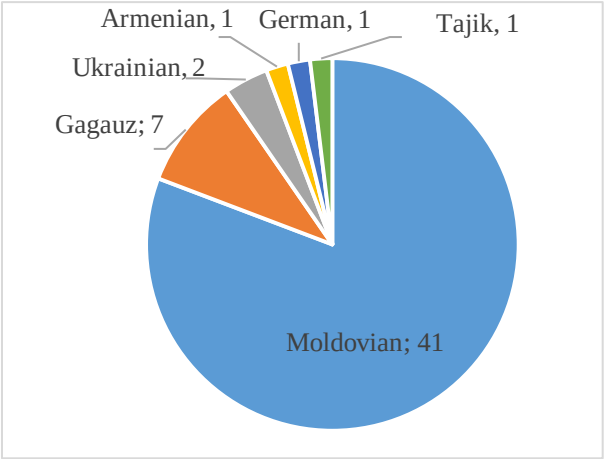

Figure 3. **Ethnic diversity of the research group**

3.2. Definition of clinical phenotypes evidenced in patients with Wilson's disease

In our cohort, this pathology is highlighted in both men and women, with the involvement of young patients, from childhood (the youngest being 5 years old, the oldest 46 years old). Thus, it is observed that in men, the neuro-psychiatric phenotype predominates, and in women there is no difference between the hepatic and neuro-psychiatric. Also, the hepatic presentation predominates in patients <20 years old, while the neuro-psychiatric one - in patients ≥20 years old. The mixed phenotype (also called neuro-hepatic) represents the most frequent type of clinical presentation in all categories (figure 4).

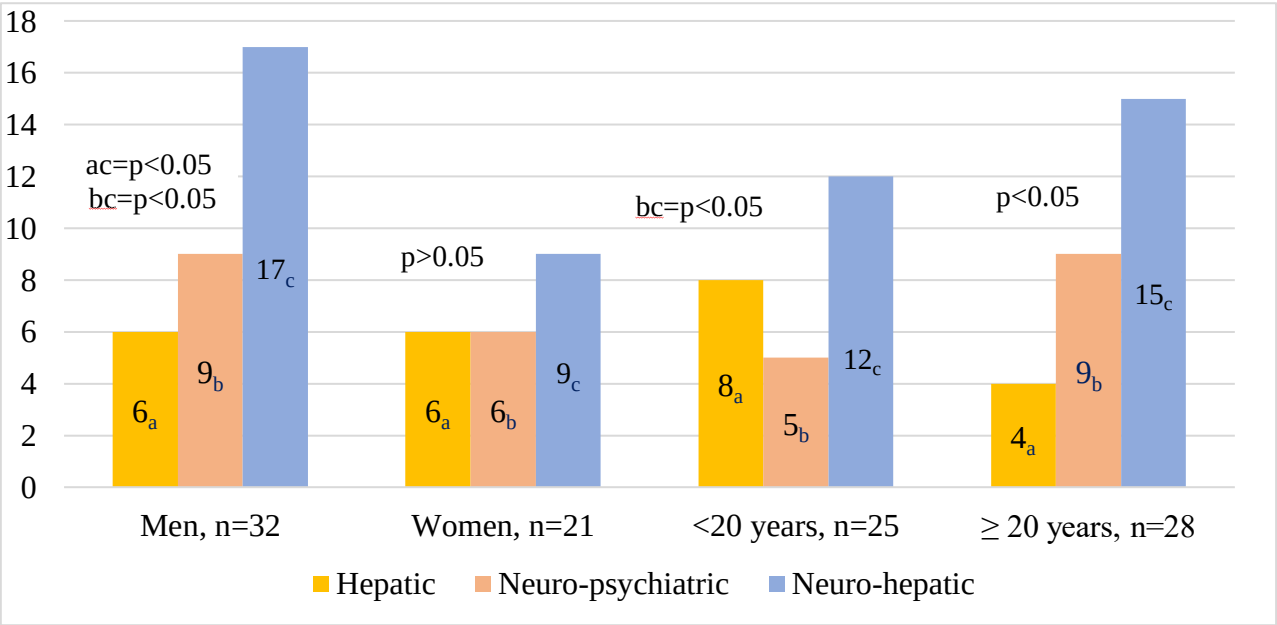

Figure 4. **Phenotype distribution by sex and age of disease onset** (n=53)

Clinical manifestations are diverse, although a stratification is observed in predominantly hepatic manifestations; or neuro-psychiatric or with mixed presentation. In this subchapter we will discuss

these phenotypes in more detail, and the clinical and genetic characteristics of the studied group according to the presentation phenotype are described in table 4.

3.2.1. Description of the hepatic phenotype

From the data obtained, we note that the patients in the research group who presented a hepatic phenotype at the onset (n=17), during the course of the disease also associated neuro-psychiatric manifestations in about 41,1%. The hepatic phenotype was present in 10 patients. The male:female ratio is 1:1. Their average age was 16,8 ± 9,81 years, the interval varying between 5 – 34 years. The clinical characteristics of this group are described in table 4. It is obvious that only in 10% the Kayser-Fleischer ring (KFR) was established, and no cerebral manifestations were identified by imaging. Biological radiography highlights the predominance of moderate cytolysis and minor cholestasis. Abnormal parameters of hepatopriv syndrome are identified in 60% of patients. Practically in half of the patients, solitary hepatomegaly is identified, and in 1/3 of the patients – splenomegaly. Liver cirrhosis was identified at diagnosis in 1/3 of the patients (of which 2 patients presented with decompensated cirrhosis). Evaluation of Cu metabolism parameters establishes the following particularities in patients with WD hepatic phenotype: most patients present low Cp, and increased urinary excretion of Cu in 24 h. While serum Cu is practically not modified. Which leads us to conclude that the parameters necessary for suspecting WD, in addition to hepato- or splenomegaly, it is necessary to evaluate serum Cp and urinary Cu excretion. All patients presented genetic mutations. The pathogenic variants *p.Phe764=* and *p.Ala1135GlnfsX13*, were reported only in the case of people with liver lesions.

3.2.2. Analysis of the neuropsychiatric phenotype

The neuropsychiatric phenotype was present in 12 patients. The male:female ratio is 1.4:1. Their mean age was 26,5 ± 9,92 years, ranging from 7 to 45 years. The clinical characteristics of this group are described in table 4. The most common clinical syndromes encountered are hyperkinetic movement disorder and pseudo-bulbar syndrome, 66,7% and 58,3% respectively. It is noted that in 25% of patients KFR was evidenced and 66,7% were identified brain imaging manifestations. No biological changes were observed in any patient. In 58,3%, patients described impaired concentration, and 50% of cases presented mood changes. Evaluation of Cu metabolism parameters establishes: most patients present low Cp, and increased urinary excretion of Cu in 24h. In 25% of patients, no

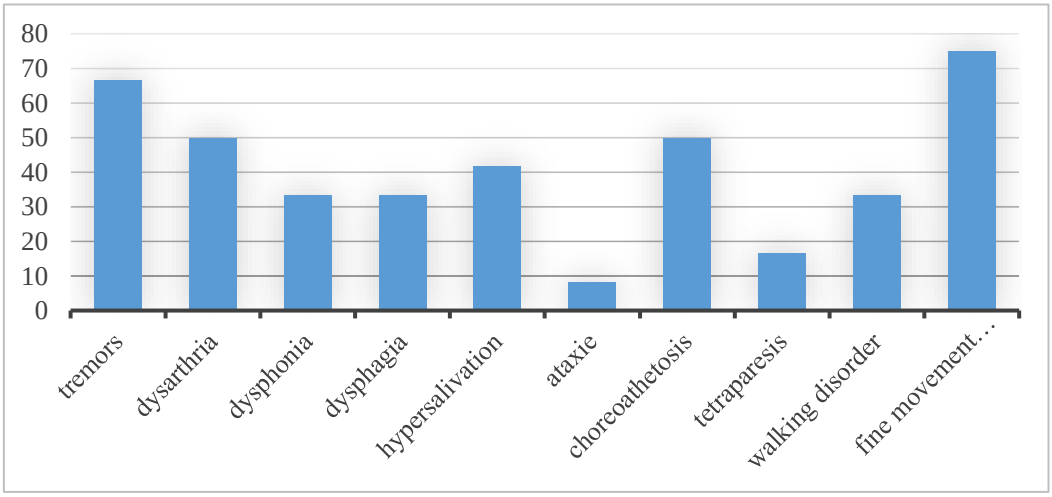

Figure 5. **Symptomatic diversity of neurological phenotype** (%)

mutations were identified. The pathogenic variants *p.T991T*, *p.E541K* and *p.Q544X* were reported only in people with neurological lesions.

The patient with neurological phenotype presents with extraordinary clinical variety, which makes it difficult to establish early diagnosis and highlight a classic clinical prototype (figure 5). The most common complaints are fine movement impairment, tremor, dysarthria and choreoathetosis.

3.2.3. Evaluation of the neuro-hepatic phenotype

The mixed phenotype (neuro-hepatic) was described in 24 patients. The male:female ratio is 2:1. Their mean age was 24,13±8,12 years, ranging from 3 to 35 years. The clinical characteristics of this group are described in table 4. Hepatomegaly and splenomegaly are identified in over 50% of cases. Liver cirrhosis was confirmed at diagnosis in 1/3 of the patients (of which 5 patients presented with decompensated cirrhosis). Hyperkinetic movement disorder was observed in 2/3 of the patients. IKF was evidenced in 62,5% of patients and 66,7% had brain lesions identified by imaging. The most common psychiatric manifestations were impaired concentration (50%) and sleep disturbance (45.8%). From the biological changes, thrombocytopenia is observed in ¼ patients and cytolytic syndrome in over ½ cases. Evaluation of Cu metabolism parameters establishes that most patients have low Cp, and increased urinary excretion of Cu in 24h. In the case of 1 patient, no mutation was identified. The pathogenic variants *c.2532delA, p.G1341V, p.Asn728Ile* and *p.A1227T* were reported only in people with neuro-hepatic phenotype.

Table 4. **Clinical characteristics of phenotypes**

Clinical parameters	**Hepatic**	**Neuro-psychiatric**	**Neuro-hepatic**
Men/women	1/1	1.4/1	2/1
Mean age at onset ± SD, years	15,2 ± 10,01*	24,25 ± 10,89*	20,64 ± 7,98
		F=2,63; p=0.08	
Mean age at diagnosis ± SD, years	16,8 ± 9,81*$_{a,b}$	26,5 ± 9,92*$_{a}$	24,13 ± 8,12*$_{b}$
median (25^{th} – 75^{th} percentile), years	15,5 (9,75 - 20)	29,5 (18,75 - 31,25)	25,5 (19,5 - 27,25)
		F=3,48; p=0.04	
Duration of diagnosis ± SD**,** months	20,5 ± 30,72	25,17 ± 30,96	30,91 ± 41,36
		F=0,305; p=0.74	
Changes in Cu metabolism, mean			
Serum Cp ± SD, (20-60 mg/dL)	10,46 ± 3,14	14,33 ± 9,96	10,61 ± 7,08
		F=1,29 p=0.28	
24h urinary C ± SD, (10-60 µg/24h)	258,71 ± 176,76*	120,52 ± 69,85*	213,03 ± 176,43
		F=1,68 p=0.11	

* statistical significance threshold between 2 compared groups: p<0.05

Table 5. **Definition of variants according to genetic parameters and phenotype**

Exon	Nucleotide modification	Amino acid modification	Variant type	Protein region	Pathogenicity	Status/ n patient	Phenotype
3		p.V456L			benign	com het/ n=2	H
4		p.S406A		MBD4	benign	com het / n=1	H
	c.1624G>C	p.E541K	missense		pathogenic	hom rec/ n=1	N
	c.1630C>T	p.Q544X	nonsense	MBD5	pathogenic	com het/ n=1	N
8	c.2183A>T	p.Asn728Ile	missense	TM3	probably pathogen	com het / n=1	N-H
	c.2292C>T	p.Phe764=	silent/ synonymous		pathogen/ probably?	com het/ n=3	H
	c.2304dupC	p.Met769Hisfs*26	frameshift	TM4	pathogenic	com het / n=5	H, N-H
10	c.2532delA	p.Val845SerfsTer28	frameshift	Domain A	pathogenic	com het/ n=1	N-H
		p.K832R			benign	com het / n=1	H
12	c.2495 A>G	p.R952K			benign	hom rec / n=1 com het / n=2	H, N-H
13	c.2972C>T	p.T991T	missense	TM6/ phosphorylation	pathogenic	hom rec / n=1	N
14	c.3207C>A	p.H1069Q	missense	ATP loop SEPHL	pathogenic	hom rec / n=16 com het/ n=18	N, H, N-H
15	c.3402delC	p.Ala1135GlnfsX13	nonsense	N-domain	pathogenic	hom rec / n=1	H
16	c.3419 T > C	p.V1140A	missense	ATP loop	benign	com het / n=1	N-H
17		p.H1207R	missense	ATP loop	benign	com het/ n=1	H
	c.3679G>A	p.A1227T	missense	N-domain	probably pathogen	com het / n=1	H
20	c.4022G>A	p.G1341D	missense	TM7	pathogenic	hom rec / n=2 com het / n=9	N, H, N-H
	c.4022G>T	p.G1341V	missense	TM7/ TM8	probably pathogen	com het / n=2	H
21		p.X1466R			benign	com het / n=1	H

Notes: com het - compound heterozygote; hom rec - homozygous recessive; H-hepatic; n – number of patients; N-neurologic; N-H – neuro-hepatic.

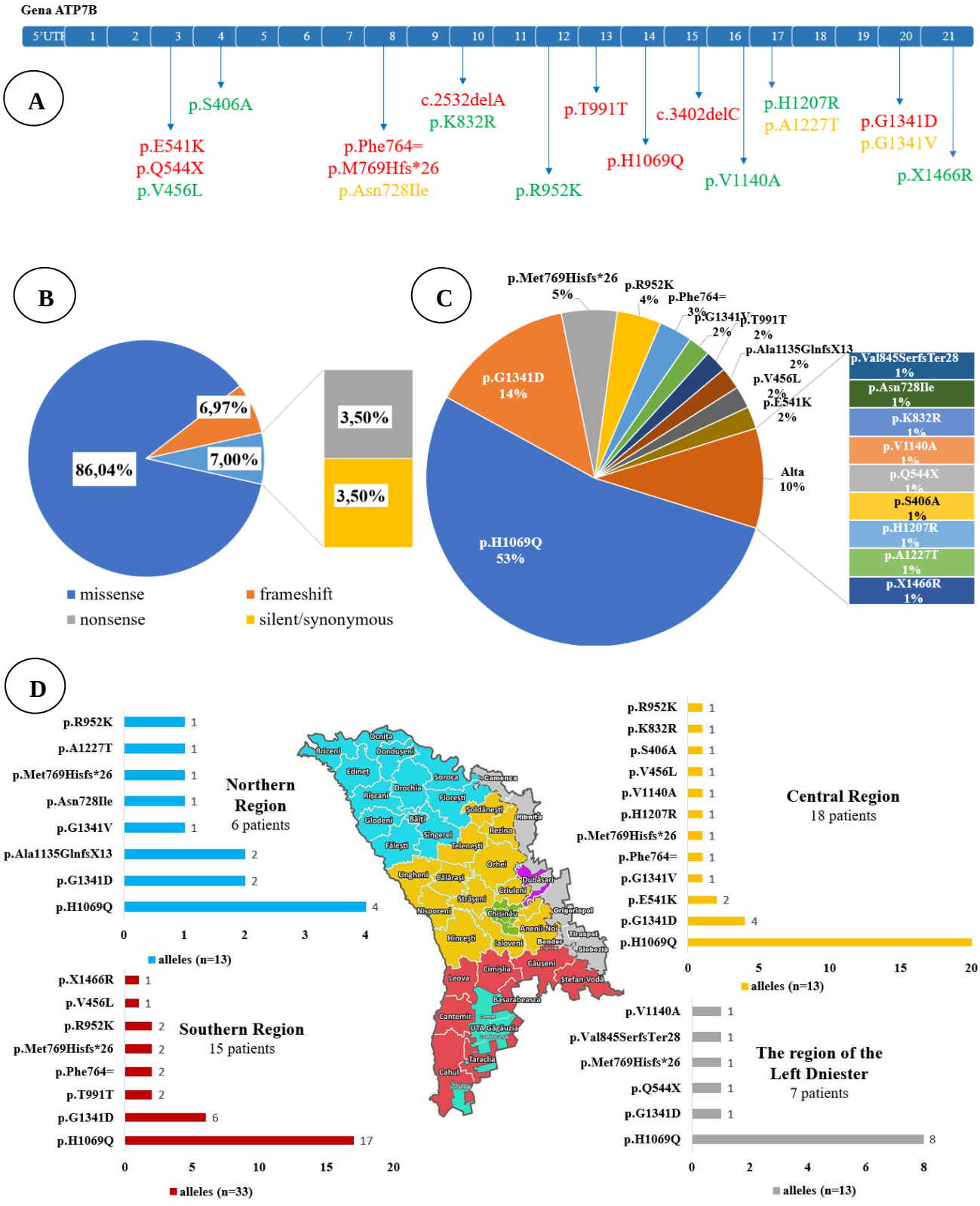

Figure 6. **Characteristics of ATP7B genetic variants identified in the study group**

A. Distribution of ATP7B variants in relation to the involved exon (note: benign – green color; probably pathogenic – orange color; pathogenic – red color); **B**. Variant types ((% of total mutations); **C.** Frequency of alleles identified in the study (% of total mutations); **D**. Distribution of alleles and patients tested according to the region of the country (n).

Thus, it was observed that the hepatic phenotype is characterized by an early onset of the disease, an early diagnosis and a statistically significantly higher Cu level compared to the other groups. The neurological phenotype presents with a later onset of the disease, a diagnosis duration of approximately 25 months, and a statistically significantly lower urinary Cu level compared to the hepatic phenotype. The mixed phenotype is highlighted by a longer duration of diagnosis. Although, the serum Cp values are different, statistical significance was not observed in any group.

3.3. Characterization of genetic mutations determined in patients with Wilson's disease

In a short period of time, in different countries from a large number of populations in patients with WD, over 900 different pathogenic ATP7B variants were identified and an unusual phenotypic differentiation was found in their combinations. It was shown that the frequency of detection and the spectrum of pathogenic variants of the ATP7B gene differ significantly in different countries. At the same time, the level of prevalence of pathogenic variants in the ATP7B gene depends not only on the geographical location of the territory of residence of the patients, but also on the ethnicity of the patient, and differences in indicators can be traced even among representatives of the same population. Pathogenic ATP7B variants can be divided into the following groups: *missense, non-sense, frameshift* and *splicing*. We aimed to characterize the genetic profile of patients in the research group, thus obtaining a genetic radiography of WD in our region.

3.3.1. Characteristics of the variants identified in the research group

Of the total number of patients in the research group (53 patients with WD), 49 were genetically tested. The PCR-SSCP method was applied to 15 patients, Sanger sequencing – to 33 patients, and the NGS method – to 1 patient (Romania). Mutations were identified in 46 patients, of which 1 single mutation – 5 patients, 2 mutations – 37 patients and 3-6 mutations – 4 patients. Most variants are of the compound heterozygous type (n=25 patients).

The research group is characterized by high mutational heterogeneity, with 19 different variants identified that were genetically and phenotypically defined in table 5 [15-19]. Variant classification was performed in accordance with the recommendations of the American College of Medical Genetics and Genomics [20]. The *p.Phe764=* variant is considered probably pathogenic, but in case of homozygosity or association with another pathogenic variant, it determines the occurrence of WD.

The characteristics of the ATP7B genetic variants identified in the studied group are shown in figure 6. Thus, it is observed that most mutations are identified at the level of exon 8. The substitution variant (*missense*) is found in 86,04%, and the most frequent allele (53%) is at the level of exon 14 – the *p.H1069Q* variant, present in 34 cases (64,1%) of the tested patients. In 16 cases (47,05%), *p.H1069Q* is found in the form of recessive homozygous, and in the other 18 patients (52,94%) it is of compound heterozygous type, of which in 12 cases the second variant is known (*p.G1341D, p.Q544X, p.V1140A, p.Asn728Ile*). *p.G1341D* is the second most frequent mutation type (15%) and is found both in homozygotes recessive (2/11 patients) and in association with other variants (9/11 patients) – *p.H1069Q* (n=7), *c.2532delA* (n=1), *p.V456L* and *p.X1466R* (n=1, same patient).

3.3.2. Identifying the genotype-phenotype relationship in patients with Wilson's disease

WD is characterized by mutational diversity, with over 900 mutations identified to date, many of which are population-specific. Establishing genotype-phenotype correlations is important for appropriate patient management, for early initiation of treatment in asymptomatic individuals to prevent certain complications, and for predicting treatment efficacy. In addition, it improves the understanding of the molecular pathogenesis of the disease. The reported associations between a given mutation and a specific phenotype in WD remain inconclusive due to several factors: the small number of patients, the large number of mutations, and the fact that most patients are compound heterozygotes, which makes it difficult to assign a clinical phenotype.

In our study, most patients with WD are compound heterozygotes (25 patients), which complicates the establishment of genotype-phenotype correlations. Another 21 patients are recessive homozygotes.

Thus, we aimed to evaluate the effect of the *p.H1069Q* mutation and other non-*p.H1069Q* mutations in the ATP7B gene on phenotypic expression in WD (table 6). The *p.H1069Q* profile included 34 patients, of whom 18 were homozygous and 16 were heterozygous. The neurological phenotype was associated with the *p.H1069Q* profile, the hepatic phenotype with the non-*p.H1069Q* profile, while the mixed phenotype is characteristic of both groups.

Table 6. **Clinico-biological differentiation related to the *p.H1069Q* and non-*p.H1069Q* profile**

		p.H1069Q	**Non-*p.H1069Q***
Patients, n		34	12
Men/women, n		18/16	8/4
Mean age at onset ± SD**,** years		19,15 ± 8,46	20,5 ± 9,85
		t=0,46; p=0.65	
Mean age at diagnosis ± SD, years		20,03 ± 8,71	24,67 ± 9,31
median (25^{th} – 75^{th} percentile), years		20,5 (14,25-26,75)	27,5 (16,25-31,75)
		t=1,56; p=0.13	
Duration of diagnosis ± SD, months		16,65 ± 20,52*	39,91 ± 39,71*
Disease onset <18 years, %		38,3%	33,3%
Phenotype, n (%)	hepatic	8 (23,5%)	4 (33,3%)
	neurologic	9 (26,5%)	2 (16,7%)
	mixt	17 (50%)	6 (50%)
		χ^2*=3,66, gl=2, p=0,16*	
KFR, %		38,3%	13,3%
Changes in copper metabolism, mean		11,73 ± 7,46	9,85 ± 7,48

	p.H1069Q	**Non-*p.H1069Q***
serum Cp (20-60 mg/dL)	*t=2,60; p=0,46*	
24h urinary Cu (10-60 µg/24h)	224,64 ± 212,77	216,21 ± 133,78
	t=0,75; p=0,89	

* statistical significance threshold between 2 compared groups: p<0.05

An interesting feature was recorded in the non-*p.H1069Q* profile. These patients were diagnosed with WD at the age of 24,67 years, and the delay in diagnosis is about 39,91 months, the predominant phenotype is the mixed phenotype followed by the hepatic one. This profile, which included 12 patients, records the longest period until the establishment of the clinical diagnosis, being also the oldest patients versus the *p.H1069Q* profile. Also, the *p.H1069Q* profile correlates with the presence of KFR in approximately 1/3 of the patients, with higher levels of Cu, a biological syndrome prevalent for pancytopenia and cholestasis. While, the other profile presents with lower levels of serum Cp, a biological syndrome prevalent for thrombocytopenia, cytolysis and hepatopriv syndrome.

The non-*p.H1069Q* profile is characterized by high genetic diversity, with rare single mutations also recorded, such as the *p.Phe764=* variant (figure 7). The inheritance of the pathogenic variant in this group is both homozygous recessive and compound heterozygous (table 7).

Table 7. **Genetic characteristic of the non-*p.H1069Q* profile**

Homozygous recessive (n=5)	**Compound heterozygote** (n=7)
p.E541K	p.A1227T
p.T991T	p.G1341D
p.Ala1135GlnfsX13	p.G1341V
p.G1341D	p.Phe764=
	p.Met769Hisfs*26
	p.Val845SerfsTer28
	p.V456L
	p.H1207R

Analyzing the *p.H1069Q* profile according to inheritance status (table 8), it was observed that the homozygous recessive subgroup is associated with a later onset, a shorter period of diagnosis and a predominantly mixed and neuro-psychiatric clinical presentation compared to the heterozygous subgroup which presents with an earlier onset, delayed diagnosis and a predominantly mixed and hepatic phenotype. In both groups, males prevail.

Table 8. **Clinico-biological picture of the *p.H1069Q* profile according to inheritance status**

		Homozygous recessive	Compound heterozygote
Patients, n		16	18
Men/women, n		8/8	8/4
Mean age at onset ± SD**,** years		22,75 ± 8,52*	15,77 ± 7,05*
Mean age at diagnosis ± SD, years			
median (25^{th} – 75^{th} percentile), years		24,0 ± 8,21*	16,5 ± 7,73*
		26,0 (18,5 - 30,5)	18,0 (9,75 – 21,0)
Duration of diagnosis ± SD**,** months		19,38 ± 23,95	14,43 ± 17,77
		t=0,68, p=0.5	
Disease onset <18 years, %		31,25%	44,5%
Phenotype, n (%)	hepatic	2 (12,5%)	6 (33,3%)
	neurologic	4 (25%)	4 (22,2%)
	mixt	10 (62,5%)	8 (44,5%)
		χ^2*=2.11, gl=2, p=0.35*	
KFR, %		50%	27,8%
		p=0.19	
Changes in copper metabolism, media			
serum Cp (20-60 mg/dL)		11,91 ± 6,86	15,59 ± 8,14
		t=1,42; p=0.17	
24h urinary Cu (10-60 µg/24h)		190,95 ± 190,97	247,96 ± 231,25
		t=0,778; p=0.44	

* statistical significance threshold between 2 compared groups: p<0.05

Also, the first subgroup correlates with the presence of KFR in 1/2 of the patients, with a lower level of serum Cp and a biological syndrome prevalent for thrombocytopenia. While, the second subgroup presents with a higher level of Cu, a biological syndrome prevalent for thrombocytopenia, cytolysis and cholestasis. From a genetic point of view, compound heterozygotes are associated with different variants: *p.G1341D, p.Met769Hisfs*26, p.Phe764=, p.V1140A, p.Asn728Ile, p.Q544X* and *p.G1341V*. These clinico-biological differences emphasize the

involvement of epigenetic and environmental factors, as well as the way in which mutations interact or influence each other.

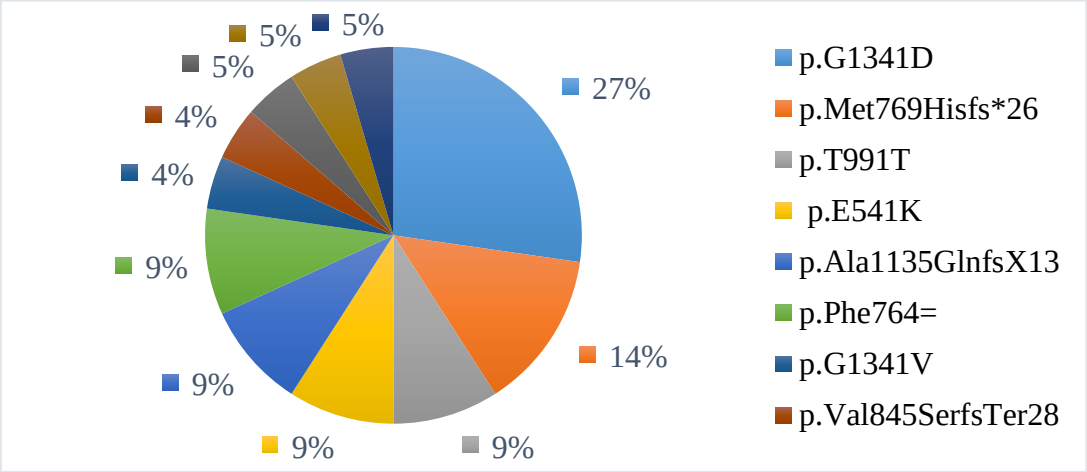

Figure 7. **Genetic diversity of the non-p.H1069Q profile**

Another characteristic is the predominance of the *missense* mutational variant, followed by *frameshift, nonsense* and *silent/synonymous*. Thus, we divided the patients into 2 groups, depending on the type of variant identified and their associations, excluding patients in whom the 2nd mutation was not detected (table 9). The first group (MMs/MMs) includes only *missense* mutations. Severe mutations (MS) include *nonsense* and *frameshift* variants, associated in the literature with a severe clinical phenotype, as a result of the synthesis of an afunctional protein. Group 2 (MS/other-M) includes MS in association with other types of mutations (other-M) – *missense*, *silent/synonymous*, *nonsense* and *frameshift*.

Table 9. **Clinico-biological characteristics related to the type of genetic variant**

		MMs/MMs	**MS/other-M**
Patients, n		32	7
Men/women, n		17/15	5/2
Mean age at onset ± SD, years		19,74 ± 8,81	20,29 ± 10,55
		t=0,15; p=0.89	
Mean age at diagnosis ± SD, years		21,03 ± 9.02*	23,43 ± 10,61*
median (25^{th} – 75^{th} percentile), years		21,0 (13,5-28)	26,0 (16,0-32,5)
Duration of diagnosis ± SD, months		22,85 ± 24,72	16,28 ± 30,07
		t=0,61; p=0.54	
Disease onset <18 years, n (%)		11 (43,75%)	3 (42,86%)
		p=0.96	
Phenotype, n (%)	hepatic	5 (15,63%)	3 (42,86%)

	MMs/MMs	MS/other-M
neurologic	8 (25,0%)	1 (14,29%)
mixt	19 (59,37%)	3 (42,86%)
	χ^2=2,64, gl=2, p=0.27	
KFR, n (%)	13 (40,63%)	2 (28,57%)
	p=0.96	
Changes in copper metabolism, mean		
serum Cp (20-60 mg/dL)	10,27 ± 5,92	9,78 ± 6,69
	t=0,19; p=0,84	
24h urinary Cu (10-60 µg/24h)	233,02 ± 203,6	170,19 ± 234,72
	t=0,72; p=0,48	

* statistical significance threshold between 2 compared groups: p<0.05

Important differences were established depending on the type of mutation established in the research group. Thus, comparing the MMs/MMs and MS/other-M groups, a statistical difference was observed between the mean age at diagnosis (p<0.05), while the duration of diagnosis recorded a visible difference in the mean values; a statistical difference in this case was not determined (t=0.61; p=0.54).

The MMs/MMs group is characterized by an earlier onset of symptoms (19,74 ± 8,81), the predominance of the mixed and neurological phenotype, the presence of KFR in 40% of patients, higher mean values of serum Cp (10,27 ± 5,92) and urinary Cu in 24 h (233,02 ± 203,6). The MS/other-M group is characterized by a later onset of symptoms (20,29 ± 10,55), the predominance of mixed and hepatic phenotype, the presence of KFR in 1/3 of patients, lower mean values of serum Cp (9,78 ± 6,69) and urinary Cu in 24 h (170,19 ± 234,72). In both groups, males prevail and an onset before 18 years of age in over 40% of patients.

3.4. The role of family screening in patients with Wilson's disease

3.4.1. Description of the results obtained in family screening of patients with Wilson's disease

Family members of a patient newly diagnosed with WD must be clinically and paraclinically evaluated, because being a hereditary disease, it can evolve asymptomatically or with a non-specific clinical picture in other relatives (siblings, descendants, parents). Thus, 13 families were examined in order to identify a-/presymptomatic members. Most of the probands are originally from the south of the country (7/12 patients), the rest come from the north (3) and the center of the country (3). In 10/12 families, first-degree relatives were tested - parents and siblings, in the other 3 cases only their descendants were evaluated. In 8 cases, both parents are healthy carriers; in the other 3 families, one parent is a healthy carrier, but the other parent was not tested. 4 siblings were identified as healthy carriers, 2 others reported healthy (no mutation was detected). The most common variants were *p.H1069Q* and *p.G1341D*, both as homozygous recessive and heterozygous (simple and compound),

a rare mutation - *c.2292C>T* was also detected. This variant causes an alternative splicing, which is predicted to lead to an in-frame deletion of 78 residues of the ATP7B protein. The conclusion of the researchers from the UK is that this mutation is pathogenic and can cause the disease [21]. The patient who presents this mutation, is with the missense variant, and presents the obvious hepatic phenotype, is from a family with an established sister with WD in adulthood. The patient has 2 children, both present with the missense mutational variant *p.H1069Q*, in compound heterozygous state, hepatic phenotype.

As a result of the evaluation in 6 out of 13 families involved in the study, 8 new members with WD were identified (6 males and 2 females). The mean age was 16,25 ± 9,7 years (range 5-34 years). In 5 cases there were first-degree relatives (siblings), and in 3 cases - second-degree relatives (cousins, nephews). 7 patients were asymptomatic, and 1 had neurological symptoms, but they were not included in any nosology up to that point. In the case of a single family, a paradoxical transmission of the disease was identified - pseudo-dominant inheritance. In 4 cases in which both parents are healthy carriers, both children were diagnosed with WD, one member being diagnosed as a result of family screening. In 6 cases, WD was associated with liver damage, and in 2 cases a mixed phenotype was reported. In 2 asymptomatic patients, no changes in Cu metabolism were observed, only cytolysis with hepatosplenomegaly or only hepatomegaly were evident, but after the D-penicillamine stimulation test, urinary Cu in 24 hours increased by more than 5 times the upper limit of normal. In both cases, genetic testing confirmed the presence of 2 pathogenic mutations. KFR was observed in 2 patients, who also presented neurological manifestations. All newly diagnosed patients with WD initiated specific therapy.

3.4.2. Creating the diagnostic algorithm in Wilson's Disease

The lack of high-quality randomized clinical trials is a common challenge for rare diseases, including WD. Therefore, recommendations in WD have been based less on data from randomized trials, and more on case series and expert consensus to complement the limited data from available studies. In addition, the phenotypic variability of WD, multisystem involvement, makes it more complicated to develop a comprehensive guideline for diagnosis and treatment. Currently, 5 international guidelines are known, mostly focused on the hepatic presentation in WD. Although they are comprehensive in terms of hepatic problems, they are limited in terms of consensual care for neurological and psychiatric manifestations of WD and in terms of certain special circumstances. In our country, the National Clinical Protocol: Wilson's Disease in Children (https://msmps.gov.md) was developed in 2016. It is the only document with recommendations for conduct in the case of a child suspected of WD. The revision of this protocol is necessary, given the outdated recommendations and the need to update the diagnostic and treatment algorithm.

Considering the results obtained in the cohort of patients with WD, we aimed to create the diagnostic algorithm based on both the current recommendations of international and regional specialized societies. The development of the diagnostic algorithm brought together the key elements of our research, namely the evaluation of WD suspects through the modified Leipzig score in 2019 (from our point of view, it is the most successful option, to be implemented in countries where there are no methods for quantifying Cu in liver tissue); stratifying patients according to the score obtained; thus, those who had a score of 1 or less will be redirected for evaluation of another diagnosis, those with a score of 2-3 will receive a probable qualification and will undergo genetic testing (according

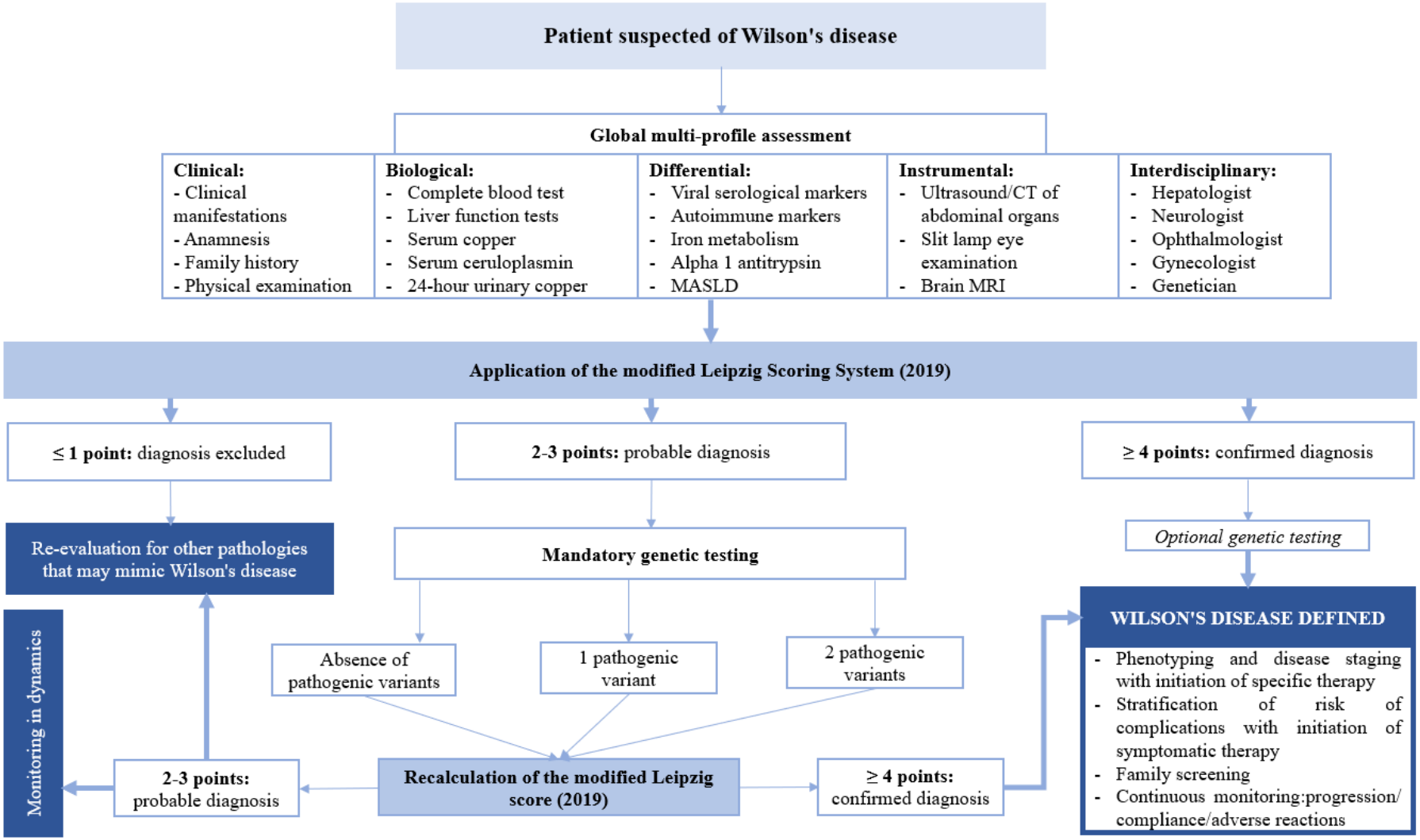

Figure 8. **Algorithm for establishing the diagnosis of Wilson's disease**

Notes: CT – computed tomography; MASLD – metabolically associated fatty liver disease; MRI – magnetic resonance imaging**.**

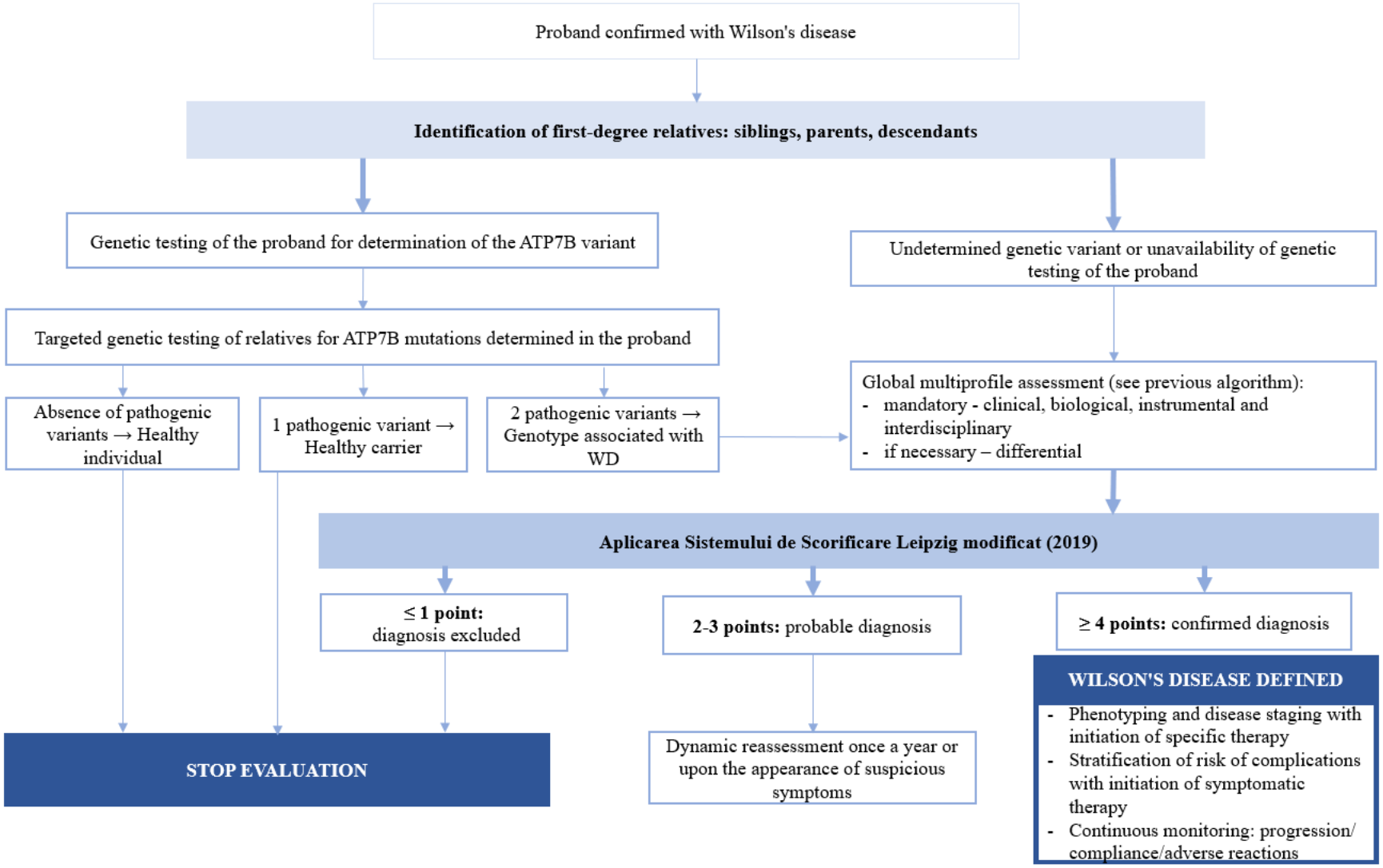

Figure 9. **Familial screening algorithm in Wilson disease**

to the Sanger method), those with a score of ≤4 will obtain a definitive diagnosis of WD, and will undergo individualized therapy, and optionally genetic testing will be recommended.

Our proposed algorithm selects individualized patient management based on clinical and genotypic phenotyping (figure 8). Another perspective is presented by the family screening algorithm in WD (figure 9), which is a mandatory component according to current recommendations

4. GENERAL CONCLUSIONS

1. The demographic aspects of the patients with WD in our study reveal a predominance of men (60.4%), a mean age of 22,62 ± 9,63 years and neurological presentation at the onset of the disease, the delay in establishing the clinical diagnosis being 26,8 ± 35,77 months.

2. The mixed phenotype represents the most frequent type of clinical presentation. In men, the neuro-psychiatric phenotype predominates, and in women there is no difference between the hepatic and neuro-psychiatric ones.

3. A high variability of genetic mutations is identified in the research cohort, with the compound heterozygous state predominating. The *p.H1069Q* mutation present in about 64% of patients. In 1/3 of the patients studied, other non-*p.H1069Q* mutations (36%) were identified that could influence the clinical diversity in WD.

4. The neurological phenotype was associated with the *p.H1069Q* profile, and the hepatic one with the non-*p.H1069Q* profile. Severe mutational variants *(frameshift/nonsense)* were associated with the hepatic and mixed phenotype, a severe evolution of the liver disease, a lower level of serum ceruloplasmin and urinary copper in 24 h.

5. Family screening of patients identified new members with WD, with a mean age of 16,25 ± 9,7 years, with the predominance of the hepatic phenotype and the *p.H1069Q* mutation. In 2 of the patients, a very rare mutation *p.Phe764=* was detected.

6. The algorithm for establishing the diagnosis of WD is important for the individualized management of the patient based on the clinical phenotype and genotype, while the family screening algorithm allows the identification of patients in the a-/presymptomatic phase of the disease.

PRACTICAL RECOMMENDATIONS

1. In all patients with a non-viral etiology liver disease with or without neuropsychiatric symptoms, regardless of age, copper metabolism parameters (serum ceruloplasmin and 24-h urinary copper) should be performed.

2. Patients identified with changes in copper metabolism indicators (serum ceruloplasmin and 24-h urinary copper) are recommended to be evaluated using the modified Leipzig scoring system.

3. Patients suspected of WD with a Leipzig score of 2-3 points are eligible for genetic testing, while patients who have met ≥4 points can be established with the diagnosis of definite WD, genetic testing not being mandatory in this case.

4. In order to optimize the management of the evaluation of the patient suspected of having WD, the "Wilson's disease diagnosis algorithm" is recommended for implementation, proposed to clinicians (family doctor, pediatrician, gastroenterologist/hepatologist, neurologist, geneticist) from the Republic of Moldova, developed in accordance with the local conditions of activity.

5. For the examination of family members of newly diagnosed patients with WD, the use of the "Wilson's disease family screening algorithm" in clinical practice is proposed. Thus, people with WD will be identified in the presymptomatic phase of the disease, which will allow the initiation of chelator therapy and the prevention of the development of complications associated with WD.

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of Ms. Cumpata Veronica, made for the doctoral thesis in medical sciences, with the topic: "Interrelation between clinical phenotype and genotype in patients with Wilson's disease", Internal Medicine Specialty (Gastroenterology and Hepatology), "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova

SCIENTIFIC WORKS

- **Articles in accredited national scientific journals:**

- articles in category B journals:

1. **Cumpătă V**. Rolul proteinei ATP7B în homeostazia cuprului și în dezvoltarea bolii Wilson: revista literaturii [The role of ATP7B protein in copper homeostasis and in the development of Wilson's disease: a literature review]. In: *Arta Medica*. 2022; 82(1): 16-22. e-ISSN 1810-1879.
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- international:

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CUMPATA Veronica

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