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RESEARCH ARTICLE

Epidemiological study on a Wilson disease group of patients

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Wilson disease is a disorder of copper metabolism caused by genetic mutations in the ATP7B gene which lead to the accumulation of copper in the body. This study was conducted using an online questionnaire consisting of 32 questions in a group of patients suffering from Wilson disease. The study included 67 people aged 7 to 56 with Wilson disease. The most common symptoms of the participants were hepatic and neurological in 22 cases (32.8%). The most common neurological symptom in the assessed cases was muscle stiffness (49.5%), followed by tremor (45%), and speech disorders (40.5%). The Kayser-Fleischer ring was present in 50.7% of the participants. The most commonly used drug treatment in the participants was D-penicillamine (77.61%).

Keywords: Wilson disease, Romania, symptoms, Kayser-Fleischer ring

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Introduction

Wilson disease (WD), or progressive hepatolenticular degeneration, is a rare autosomal recessive genetic disorder in copper metabolism and consists of copper accumulation in multiple organs (liver, cornea, brain). *ATP7B* gene expression is the process by which the genetic information contained in the ATP7B gene is transcribed and translated into a functional protein - ATP7B, a copper-dependent ATPase. This protein plays an essential role in copper homeostasis, especially in the liver. *ATP7B* also facilitates the transport of copper in the bile. The genetic defect leads to dysfunction of this transporter, thus copper accumulating in the cell results in its destruction. In the absence of the proper treatment, free copper enters the bloodstream and accumulates in the organs. Systemic copper storage has a varied phenotype, from asymptomatic forms to liver and neuropsychiatric damage [1].

Positive diagnosis is, in most cases, delayed or overlooked due to the variety of symptoms and signs. Due to misdiagnosis or a prolonged initial period of silence, WD is often detected too late. A delayed diagnosis is a key risk factor for a poor treatment outcome. There is great variability of symptoms in WD that most commonly present between the ages of 5 and 35 years.[2]

WD results from mutations in the *ATP7B* gene that is designed to synthesize the copper transporter protein. As a result of these mutations, an imbalance in copper metabolism occurs, leading to the impossibility of removing it from the body and accumulation of excess levels in the cell. The transport of copper to the intestine involves several specific and non-specific transporters located in the enterocytes of the small intestine, especially in the duodenum. Two of the most important copper transporters are: *CTR1* (Copper transporter 1), the main copper transporter, encoded by the *SLC31A1* gene, and *DMT1* (Divalent Metal Transporter 1), non-specific transporter, known as *Nramp2* or *SLC11A2*. In the intestinal lumen, ingested copper (usually in the form of Cu^{2+}) is reduced to Cu^{+} of enzymes such as metal reductase. Cu^{2+} is absorbed by *CTR1* in enterocytes. In some conditions, Cu^{2+} can be transported by *DMT1*. Once inside, copper is bound to intracellular chaperones (e.g. ATOX1) carrying it to *ATP7B*, involved in the export of copper into circulation. [3].

In the liver cell, under physiological conditions, the copper coming through the portal route enters the cell through copper transporter *CTR1* located at the apical pole. Thus, copper enters the cytosol, where it binds to the chaperon protein *ATOX1*. This transports copper to *ATP7B*, a transporter that sends the copper molecule into the Golgi apparatus. There it is incorporated into apocerulo-plasmin, which has 8 copper binding sites. Thus, apocerulo-plasmin becomes ceruloplasmin, which will transport the copper molecules back to the plasma through the latero-basal pole. An alternative route would be the exocytosis of copper unattached by ceruloplasmin (excess copper), through the canalicular membrane into the bile. The bile duct is vital for controlling hepatic copper levels. Moreover, *ATP7B* acts as a conveyor here. [4]

Globally, WD prevalence can vary significantly and has increased over time due to much more effective diagnostic methods, with recent studies suggesting values between 1:40,000-1:60,000. In Europe it is estimated to be around 1:40,000-1:50,000, with dominant areas such as Sardinia with a prevalence of 1:16,000. The highest incidence in the world was reported in Costa Rica (4.9 per 100,000 inhabitants). In Europe, the disease is more commonly di-

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agnosed in Germany (2.5 per 100,000 inhabitants) and Austria (3.0 per 100,000 inhabitants) [5].

Studies conducted in France and Sardinia suggest that genetic prevalence could be 3-4 times higher than clinical prevalence. The prevalence in France is estimated to be 1:63,000, in Taiwan 1:55,000, and in Hong Kong 1: 40,000, but, nevertheless, there have also been overlooked cases. In Taiwan the female/ male ratio is 1/1.75 suggesting a number of undiagnosed cases among female populations because the female/ male ratio is 1 in other patient groups. In the French study, prevalence was higher in the younger age group, near large cities, the highest age group being 20–29-year-olds (1:37,000) [6]. In addition, a study conducted in France found that 1 in 31 is a heterozygous carrier [7], corresponding to a prevalence of 1:1000 births. The discrepancy between the frequency of heterozygous carriers and the prevalence of WD suggests incomplete penetrance of the disease, but further studies are needed [8]. The factors contributing to this discrepancy include the body's particular response to copper metabolism, epigenetic factors or misdiagnosis with another metabolic syndrome [9] (Figure 1).

Epigenetic changes are caused by environmental factors such as diet, stress, exercise, toxins. These can diminish or exacerbate the clinical presentation and progression given by the accumulation of copper in WD (Figure 2).

Our interest in this topic is due to the fact that the condition is understudied both nationally and globally. Romania lacks a diagnosis protocol and database for this disease. The fact that the condition is understudied nationally makes it an area of important research interest. We also believe that this study will have a positive and innovative impact on the national literature, being the first study in Romania that aims to analyze this condition from an epidemiological point of view. The aim of the present study is to investigate the severity of WD in patients in Romania.

Material and methods

The study included 67 patients diagnosed with WD between the ages of 7 and 56 years and was conducted from September 19, 2022 to December 20, 2024. The data collection was carried out through an online form consisting of 32 questions, applied targeted on social media networks in a group with people suffering from WD. The survey included questions about demographics, signs of disease and symptoms, laboratory tests, hereditary history, any treatment administered or access to treatment throughout the year, diet, other opinions or comments related to the par-

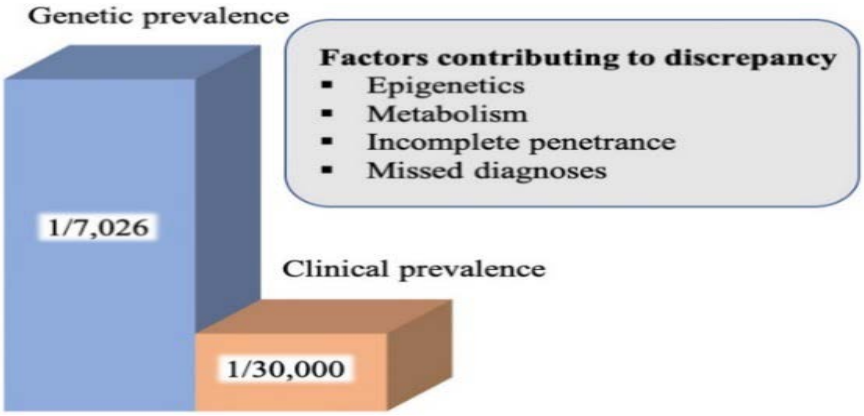

Fig. 1. The difference between clinically diagnosed and genetic prevalence [9]

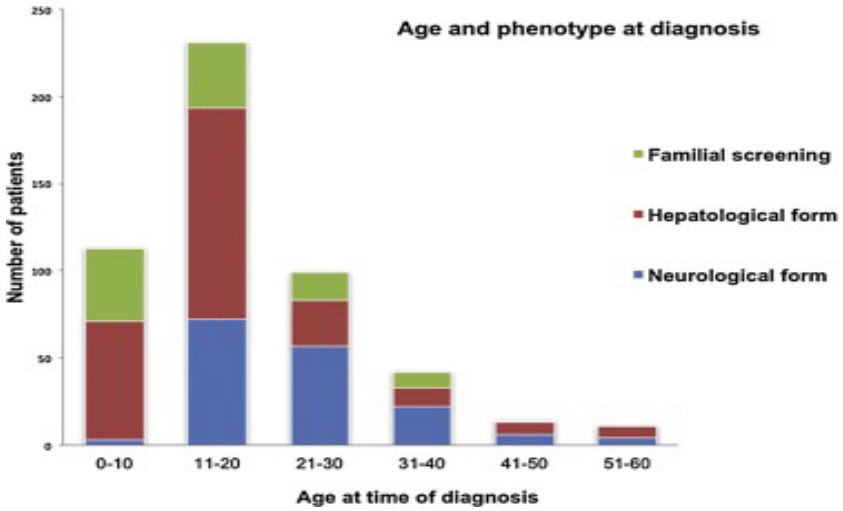

Fig. 2. Age and phenotype at WD diagnosis according to a study conducted in tertiary centers in Greece over the past 30 years [10]

ticularity of the respondent's case. Anonymity and confidentiality of responses were ensured. The images in this article come from two patients who agreed to the publication.

Results

Table 1 lists the demographic characteristics and symptoms of respondents.

The hereditary history, investigation and treatment methods of the participants are mentioned in table 2.

Table 3 lists associations of symptoms in study participants.

Some testimonials of study participants suffering from WD will be included below. These testimonials encompass the burden of the disease, the everyday problems that patients face.

"I would like to mention that as soon as I was diagnosed with WD, I was given cuprenil, but my symptoms worsened, my tremors got stronger, I could barely speak and walk, I had no control over my saliva, which made me stop the treatment. In 1992 I managed to be hospitalized in a clinic in Germany, here I was given metal-captase (D-penicillamine 300 mg) in combination with zinc and vitamin B6, this combination brought fantastic results in a year.

"There were times when I did not have easy access to treatment."

„I can't find doctors who want to take care of me because they don't have the necessary skills. They send me from one to the other. They even told me it was a miracle that I was stable and that it was the wrong diagnosis. I stayed with the same treatment as 7 years ago because as an adult I did not find a doctor who wanted to get involved."

"There is no treatment in all the cases and genetic testing is not done for free as it is done in other countries. Being a rare disease, genetic testing for it should be free."

"Receiving the treatment is very difficult because it is not provided in the country and when the pharmacies in the city receive the medication they prefer to say that they do not work with the distributor who has them. So I buy the treatment from Spain."

"In the year 2000-2001 in Romania, genetic tests for the detection of the disease could not be performed. Ceruroplasmin and cupruria were not easy to perform.

"The Ministry of Health should include the mandatory blood and urine copper test in children after they reach the age of 3, in order for early detection and the provision of more information about this disease."

"The sad thing is that I had to leave the country and move to Spain to have access to treatment and save my life."

"Cupripen is difficult to find in pharmacies, zinc and hepatoprotector should be reimbursed taking into account that it a treatment for life."

Discussions

In Romania, WD is recognized as a rare condition, but with significant public health implications, requiring early diagnosis and appropriate treatment to prevent severe complications.

Table 1. Demographic distribution and symptoms in the participants

Variables	No.	%
Sex		
Females	35	52.2
Males	32	47.8
Urban residence	40	59.7
Mean age (minimum-maximum)	20 years (7-56 years)	
Neurological symptoms		
Muscle stiffness	33	49.5
Tremor	30	45
Speech disorders	27	40.5
Sialorrhoea	26	39
Reduced facial expression	22	33
Involuntary movements of the hand	21	31.5
Kayser-Fleisher ring (yes)	34	50.7
Mood swings (no/%)	34	50.7
Depression (no/%)	42	62.7
Symptoms on onset		
Tremor	24	36
Speech disorders	12	18
Hepatic cytolysis	11	16.5
Sialorrhoea	3	4.5
Fatigue	4	6
Ascites	3	4.5
Other	5	7.5
Duration between the first symptom and diagnosis		
Under 1 year	27	40.3
1-2 years	27	40.3
2-10 years	10	15
Over 10 years	3	4.5
Menstrual disorders (yes)	19	28.4
Fertility (no/%)	50	74.6
Genetic testing children (no/%)	16	23.9
Family aggregation (no/%)	40	70.1

Table 2. Hereditary history, methods of investigation and treatment in the participants

Variables	No.	%
Degree of kinship of patients		
Parents	4	5.97
Siblings	11	16.4
Cousins	10	14.9
Other (uncle/aunt)	3	4.5
Consanguinity (no/%)	65	97.1
Laboratory tests		
Urine copper	62	92.5
Blood copper	60	90
Ceruloplasmin	60	90
Imaging	51	76.1
Ophtalmic examination	56	83.5
Genetic testing	26	38.8
Liver biopsy	16	23.8
Initial misdiagnosis (no/%)	**37**	**55.2**
Initial misdiagnoses		
Hepatitis	9	13.5
Depression	7	10.5
Liver cirrhosis	4	6
Other	9	13.4
Antidepressant treatment (no/%)	27	40.3
Impulsive behavior (no/%)	41	61.2
Anaemia (no/%)	41	61.2
Relief of symptoms		
Under 6 months	21	31.3
6 months - 1 year	21	31.3
1-2 years	9	13.4
Over 2 years	2	3
No remarkable improvement	14	20.9
Medication		
D-penicillamine	52	77.6
Zinc	28	41.8
Trientine	13	19.4
Low copper diet		
Strict diet	18	26.9
Partial avoidance	41	61.2
Medication only	8	11.9
Food restriction before treatment (yes)	43	64.2
Access to treatment (yes)	52	77.6

Table 3. Associated symptoms presented by patients

Variables	%
Hepatological	22.4
Neurological	26.9
Hepatological, Psychiatric	1.5
Neurological, Hepatological	32.8
Neurological, Hepatological, Psychiatric	7.5
Neurological, Psychiatric	1.5
Asymptomatic	7.5

There is no significant gender difference in our study with the slightly predominant female gender. One study in Serbia featured 54.9% male and 45.1% female participants. [11]

Regarding the home environment, most of the participants came from urban areas.

Because WD is a degenerative disease it is very important that the diagnosis be early, in our study the mean age of diagnosis was 20 years with the minimum age of 7 years and the maximum of 56 years, an increased mean age compared to the Sardinian study where the mean age was 15 years and 6 months with the minimum age 4 years and 1 month and the maximum 44 years [12].

The most common symptoms in study participants were related to liver along with neurological (32.8%) cases, followed by neurological damage (26.9%) cases, and liver damage (22.4%). Liver, neurological and psychiatric disorders were encountered in 7.5% cases, one case showed a hepatic and psychiatric form, and 5 cases were asymptomatic. Compared to the study conducted in Sardinia [12] where the most common symptoms were the hepatological and neurological ones with 38.23% cases, 29.41% cases had hepatic presentation and we noticed that neurological manifestations were lower compared to our study and occurred in only 10.29% cases, and 22.05% asymptomatic cases, which is 3 times higher compared to our study, as such, we can infer that there was a lack in early diagnosis in our study.

The earlier the diagnosis, the more effective the treatment. In our study 40.3% of cases were diagnosed in less

than one year, 40.3% in 1-2 years, 15% were diagnosed between 2-10 years, and 4.5% in over 10 years. A very important thing to note is that 44.8% of the participants of our study had an initial wrong diagnosis, a contributing factor to this may be the polymorphism of clinical manifestations. The most common method of diagnosis among participants was copper in the urine on 24h, with 92.5% of cases, followed by copper in the blood 90% of cases, ceruloplasmin in the blood 90% of participants, imaging studies (MRI, CT, ultrasound) 76.1% of participants, eye examination 83.5% of participants, 38.8% of participants had genetic testing, and 23.8% of participants had liver biopsy.

The only current screening method that can be performed in children is genetic testing. However, the high cost, the access to free testing, limited by bureaucracy or waiting lists, seem to be among the limits and challenges of this testing, aspects identified also in the respondents' testimonials. Although the optimal time for screening is questionable, a child's 3-year period would be the most indicated. Among the study participants who had children, only 10.4% had their genetic testing performed. Of the study participants, 29.9% still had cases of WD in the family, the most common among siblings (55%), cousins (50%), and parents (20%).

A pathognomonic sign in WD is the Kayser-Fleisher ring, correlated with the symptoms, in our study occuring in 50.7% of the participants. This was most commonly encountered in the participants with neurological and hepatic symptoms 61.7%, in those with only liver damage the Kayser-Fleischer ring was present in 44.1%, of the participants with psychiatric, hepatological, and neurological symptoms 14.7% presented the ring, in cases with only neurological damage the ring was present in 52.9% of cases, and in those with liver and psychiatric damage it was present in 2.9% of cases (Figure 3).

Regarding the fertility rate among the participants, 17 (25.4%) of the 67 participants in the study had children.

Up to 30% of WD patients may first experience psychiatric symptoms, according to epidemiological research. The earliest psychiatric symptom may present in youth with a loss of academic performance, aggressiveness, impulsive irritability, antisocial behavior. The most common signs of psychiatric disorders in WD are mood swings. Between 4% and 16% of WD patients attempt suicide, while between 20% and 60% of WD patients experience depression over the course of the disease [13].

In our study, 37.3% of participants claimed to be experiencing depression. Of those experiencing depression only 16.4% were undergoing antidepressant treatment. About half of the study participants (49.3%) claimed that they swung quickly from one mood to another, this having an unfavorable impact on the quality of life. At the same time, 38.8% of the study participants believed that they displayed impulsive behavior. All WD patients should undergo screening tests for depression and those who have psychiatric disorders should be treated with appropriate treatment. Cognitive function should be routinely assessed and psychological counseling is recommended for both the patient and their family.

The rate of ignoring the diagnosis is high in Romania, but also globally, as a result, it is important to consider WD in a child or adolescent when experiencing liver damage of the type hepatitis, liver failure and neurological symptoms such as tremor, muscle rigidity, speech disorders.

The most common neurological symptoms in the cases analyzed in our study were muscle stiffness, followed by tremor, speech disorders, sialorrhea, reduced facial expression, and the least frequently encountrered one was involuntary movement of the hand.

In a Polish cohort study, which analyzed neurological manifestations in patients with WD, the most common

Fig. 3. Complete Kayser-Fleischer ring on a blue eye (photo provided by one of the study participants)

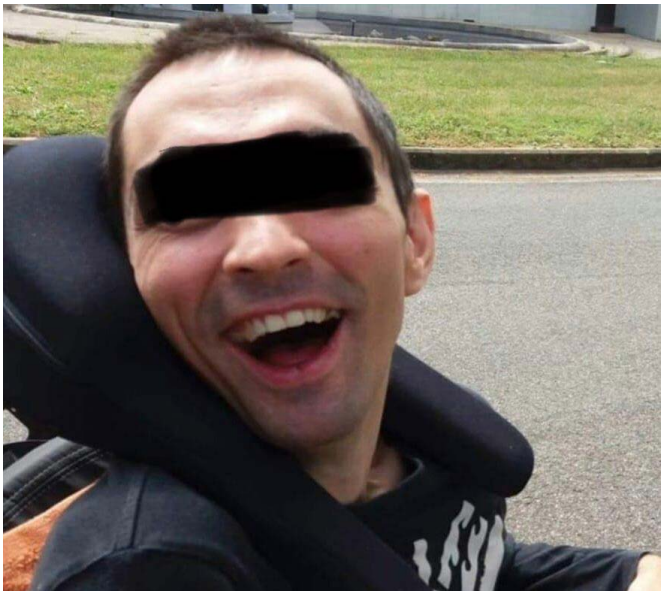

Fig. 4. WD characteristic sign of risus sardonicus in a 37-year-old man

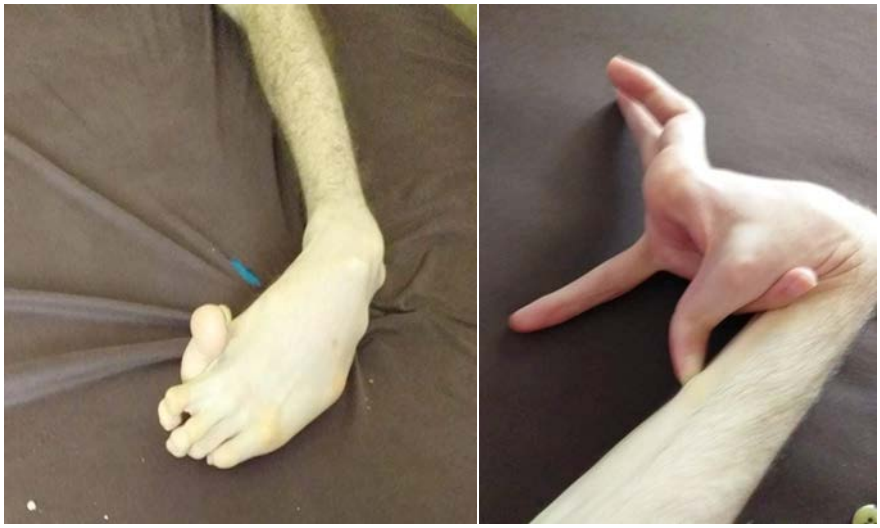

Fig. 5. Dystonia of the left upper limb and left lower limb in a patient with WD

neurological symptoms were dysarthria, followed by tremor, muscle stiffness, reduced facial expression [14].

A classic sign of WD is facial dystonia known as *risus sardonicus* or "*Wilson's face*" which consists of an excessively forced smile (Figure 4 and 5).

The images belong to our archive, supplemented by contributions from study participants who willingly granted their consent for publication.

A risk factor in the occurrence of WD is consanguinity. In patients in our study, consanguinity was present in 5 (7.5%) cases.

Treatment in WD is laborious. According to the testimonies of the participants in our study, they have difficulties in obtaining treatment, they are obliged to turn to sources from abroad, so in Romania, the Ministry of Health should permanently provide an alternative range of drugs that meet the needs to deliver the necessary quantities in the shortest term and oblige the pharmaceutical units to store medicines if they are not in stock on request. It is necessary to implement a national registry that comprises WD patients in Romania in order to have a record of the number of cases, the treatment administered, and each patient's evolution. Also, the establishment of an association to support patients in procuring the treatment, the last generation being very costly.

The first intention is the drug treatment that consists of copper chelators to which the hygienic-dietary treatment is added which consists of eliminating foods rich in copper. Symptomatic treatment is added to these. Patients with neurological suffering are recommended sessions of physical therapy, hydrotherapy, while those with speech disorders are recommended speech therapy.

The most common drug treatment used among study participants was D-penicillamine (46.3% of cases), followed by D-penicillamine and zinc (29.8% of cases), 10.4% of cases used Trientine as chelating treatment, zinc and Trientine in 6% of cases, only zinc in 6% of cases, D-penicillamine and Trientine in 1.5% cases.

In a study in France the most used treatment was D-penicillamine (30.5%), followed by Trientine (14.70%), and zinc acetate (13.0%) [7].

In Romania, 22.4% of the cases that participated in the study do not have access to treatment throughout the year. Also, in the administration of the treatment, it is important to have a lunch break for a more efficient absorption. In our study, 64.2% of the patients took this break.

In WD hygienic-dietary regime is also important and it consists of avoiding foods that have a high copper content. In our study, more than half of the cases only partially followed the dietary plan, avoiding only a few foods rich in copper, 26.9% of cases strictly followed it and analyzed everything they ate, and 11.9% did not follow the plan, considering that drug treatment was sufficient.

For a better recovery of the degree of invalidity, sessions of physical therapy, hydrotherapy, speech therapy are indicated. The goal is to improve the quality of life and maintain as much autonomy as possible for the patient, even the presence of sequelae. In our study, 28.4% resorted to recovery sessions and most (13.4%) from the time of diagnosis to the present, 9% under 6 months, 6.5% between 6 months and one year, and 1.5% resorted to recovery sessions until the symptoms improved.

Study limits. The study involved a small batch, which limits the statistical power and generalization of the results, but WD is known to be a rare condition. Also, according to the clinical expression, WD had heterogeneous manifestations among the respondents (hepatic, neurological, psychiatric), which can make it harder to homogenize the batch and compare cases. However, mentioning these aspects does not weaken the study, but gives it credibility and guides future research.

Conclusions

The severity of WD is based on many aspects, the most important being early diagnosis and access to treatment throughout the year. The prognosis is favorable with early

diagnosis and treatment, but it is crucial to diagnose individuals before they develop major symptoms. Therefore, advances in WD screening can lead to an earlier diagnosis and better results. WD is also a complex disease, the diagnosis being a challenge that requires a multidisciplinary team consisting of neurologist, gastroenterologist, psychiatrist, psychologist, ophthalmologist, pediatrician in order for the treatment to have a good outcome.

Improving treatment rates, raising patient awareness, educating patients about the long-term impact of the disease, or developing a new treatment paradigm could make a significant contribution to reducing the burden of the disease. In order to provide an early and effective preventive therapy that can significantly improve outcomes in WD patients, new biomarkers and the development of new techniques should be continuously investigated worldwide.

There are few WD studies conducted in Romania, therefore more studies are needed to analyze the complexity of this condition, studies which should result in ways to improve the patient's quality of life and offer them a life span close to that of a healthy person.

Author Contributions

IRM (Conceptualization, validation, investigation, resources, writing—original draft preparation, project administration); TSV (Conceptualization, methodology, formal analysis, writing—original draft preparation); MAB (validation); LV (validation). All authors have read and agreed to the published version of the manuscript."

Conflicts of Interest

None to declare.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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