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Phenotypic polymorphism in Wilson's disease – between genetics and epigenetics

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Abstract

Background: Wilson's disease is a rare, autosomal recessive genetic disorder that affects the biliary excretion of copper and its toxic accumulation in various tissues, especially the liver and brain. It is widespread throughout the world, with a high prevalence in socio-culturally isolated communities. The course of the disease and the age of onset depend on the site of mutation in the gene and the degree of functional impairment of the ATP7B protein. The presence of the compound heterozygous patient complicates the comparative genetic and clinical evaluation. Therefore, it is necessary to analyze Wilson's variants in both the homozygous and the compound-heterozygous conditions to better understand the genotype-phenotype correlations and the incomplete penetrance observed in this disorder. Outlining clear phenotype-genotype associations is difficult due to a large number of mutations and different clinical presentations, but the involvement of epigenetic factors, modifying genes, environmental and lifestyle factors could explain the differences in evolution and onset in members of the same family and not only.

Conclusions: Wilson's disease is a genetically and clinically complex disorder. Although the results of genotype-phenotype correlation studies are not well defined, and in some cases are completely contradictory, some peculiarities related to the age of onset, sex, clinical phenotype, and the evolution of the disease have been highlighted. The interaction between genetic mutations and epigenetic factors may explain the phenotypic variability, but needs further study.

Key words: Wilson's disease, ATP7B gene, mutation, genotype-phenotype correlation, epigenetic.

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Introduction

Wilson's disease (WD) is a monogenic disease with an autosomal recessive transmission, caused by a disorder of copper (Cu) metabolism. Reduced excretion of Cu leads to its accumulation in tissues, and toxic deposits induce oxidative stress, alter gene expression, inhibit directly protein activity and affect mitochondrial function, causing multiple structural and functional damage in several organs, especially the liver and brain [1, 2].

The disease manifests as a multisystemic condition with an unpredictable clinical picture, and the symptoms appear depending on the most affected organ. The severity of the disease depends on the site of mutation in the ATP7B gene and the functional capacity of the ATP7B protein [3]. WD may have an asymptomatic or silent course (no symptoms: apparently good), which makes it difficult to make a diagnosis. Due to dysfunction in many organs, clinical and laboratory features are often subtle and may mimic alternative diagnoses, and in the process of evaluating a patient with a variety of signs, symptoms, and laboratory abnormalities, a differential diagnosis with WD is necessary [4].

The definitive diagnosis can only be established by performing specific tests for WD, and using the Ferenci score (Leipzig, 2001) facilitates the process of assessing a suspected patient of WD [5]. For a patient with a defined clinical diagnosis of WD, molecular-genetic analysis is not mandatory but may provide the information needed to guide relative screening, and knowledge of mutational status may help assess genotype-phenotype correlation about demographic, clinical, and paraclinical indices, as well as in new treatment technologies, e.g. gene therapy [6].

The publications were selected from the PubMed and HINARI databases – Research4Life program, using search terms in English: *"Wilson's disease", "ATP7B gene", "ATP7B mutation", "genotype-phenotype correlation", "Wilson pathogen variant", "epigenetic in Wilson's disease", "prevalence", "phenotypic diversity"*. According to the search criteria, all full-text publications offered by these platforms were selected, and articles in English have been prioritized since 2002, although no language limits have been set. Sources also include articles published in the Republic of Moldova and Romania. After a preliminary analysis of the titles, in the final bibliography were selected original articles, narrative synthesis, meta-analyses, systematic reviews, series of cases relevant to the research topic, book chapters, which addressed epidemiological data and global distribution of

the disease, the role ATP7B gene and mutations associated with WD development, the correlation between genotype and phenotype, the involvement of genetic and epigenetic factors in the evolution of the disease. Articles on clinical and paraclinical diagnosis, current therapies, new diagnostic and treatment methods, scores, and questionnaires used in WD were excluded.

Discussion

After processing the information from the PubMed and HINARI databases, according to the search criteria, 38 relevant sources were selected, which were considered representative of the material published on the topic of this synthesis article. Publications, the content of which did not reflect the subject matter in this article, although they were chosen by the search engine, as well as the articles, which were not accessible for free viewing and through the HINARI database (*Health Internet Work Access to Research Initiative*) or available in the medical scientific library of *Nicolae Testemitanu* State University of Medicine and Pharmacy were excluded from the list.

Epidemiological data. Wilson's disease is a worldwide genetic disorder [7]. Epidemiological estimates have established significant differences between the clinical and genetic prevalence of the disease, and this is due to several factors, including genetic, epigenetic modifiers, and habits (diet, traditions, exposure to toxic environment) [4]. Incidence varies between 1: 5000 and 1: 30000, but is considered to be more common than previously thought [5, 7]. The frequency of carriers in the general population varies from 1: 90-150, which makes it one of the most common Mendelian disorders [1]. The prevalence of the disease is higher in socio-culturally isolated communities, as well as in populations where marriages between relatives are practiced (consanguinity) [7, 8]. In Europe, the clinical prevalence is estimated at 12-20: 10000, while the prevalence of the affected gene is higher at 1: 7000 [9]. The age of onset of WD varies from 2 years to 80 years, but most are diagnosed between 5-35 years [5, 7]. The frequency and distribution of ATP7B mutation in the Republic of Moldova are not known for sure.

ATP7B gene: structure and role. The ATP7B gene is located on the long arm of the 13th chromosome (13q14.3). It consists of 20 introns (non-coding nucleotide sequences) and 21 exons (coding nucleotide sequences) and has a genomic length of about 80 kb. It is mostly expressed in the liver but can be found in the kidneys, placenta, mammary glands, brain, retina, and lungs [10, 11]. The gene encodes the structure of a Copper-transporting P-type ATPase, officially called ATP7B, which carries Cu intracellularly, and mutations in the gene cause the synthesis of a defective or dysfunctional protein. Currently, ATP7B is the only gene known to cause WD [12]. The ATP7B protein, also called Wilson ATPase, is a protein with a complex multi-domain structure, with an essential role in biosynthesis and in maintaining Cu homeostasis in the body. In each functional domain of the enzyme, there are unique amino acid sequences ("motif"), with a major impact on the process of transporting Cu, and mutations in their level are associated with the development of WD [11]. The biosynthetic function is achieved by incorporating Cu into the apo-ceruloplasmin (the main substrate that receives Cu from Wilson protein), and after the acquisition of 6 Cu atoms, the "mature" ceruloplasmin is released into the systemic stream. The function of maintaining Cu homeostasis is achieved due to the ability of the protein to rapidly relocate from the Golgi complex to the biliary pole of the hepatocyte, in response to changes in the level of Cu in the cell, to eliminate excess metal in the bile [2].

Transmission and inheritance. WD is a monogenic disorder with autosomal recessive transmission according to the Mendelian distribution. This means that to develop the disease it is necessary to inherit 2 affected genes, from each parent one (recessive homozygous), and the one who inherits only one mutated gene becomes a healthy carrier or simple heterozygous, respectively he will not develop the disease, but will pass on the gene mutation to his children [9]. Compound heterozygotes are people who have a different mutation on each chromosome, while recessive homozygotes have the same type of mutation on both chromosomes. Thus, the disease is found in recessive homozygotes and compound heterozygotes, the latter being the most common of WD [12]. It is extremely important to make the differential diagnosis between compound homozygotes/heterozygotes and simple heterozygotes (healthy carriers), especially at the asymptomatic stage, because for patients in the first group it is recommended to initiate chelating agent therapy as early as possible to prevent while healthy carriers will not develop WD and do not require the administration of specific preparations, which could cause potentially serious side effects [13].

Despite Mendelian transmission of the disease, some deviations have been observed. It is unusual for autosomal recessive mutations to occur in several consecutive generations, but in WD this is due to the high frequency of healthy carriers [9, 10]. Thus, Poujois A. and Woimant F. [9], Chang I.J. and Hahn S.H. [10] reported cases of "pseudo-dominant" inheritance, which led to the screening of the entire family. Given that WD is a treatable disorder, it is insufficient to inform only about the possible risk of genetic disease in the family, so a complex family screening is recommended. In the first stage, the siblings are checked, because their risk of having two mutations is 25%. The second stage will examine the descendants (0.5% risk), then the parents, uncles, aunts, and grandchildren [9].

In a significant number of clinically diagnosed cases of WD, the mutation of the ATP7B gene failed to be detected, and this highlighted the genetic heterogeneity of this condition, but also suggested the presence of paradoxical mutational mechanisms. Thus, Coffey A.J. and colleagues [14] in the genetic study of patients with WD in the United Kingdom identified patients with atypical patterns of inheritance that may cause WD, such as single-parent segmental isodisomy.

The model results when both homologous chromosomes are from the same parent and represent a rare accidental error in the process of chromosomal segregation during meiosis [14]. This is important for clinical practice and genetic counseling, as genotyping of asymptomatic parents or obtaining complete sequencing of the ATP7B gene will be considered to confirm the pathogenic variant [12].

Pathogenicity of mutations. Mutations can occur anywhere in the gene – exons, splicing site (intron/exon boundary zones), introns, untranslated regions 5 '(UTR5) and 3' (UTR3), and promoter. The most common mutations are located in the 8th exon (p.R778L) and the 14th exon (p.H1069Q). However, there have been reports of people diagnosed with WD based on clinical and biochemical tests, but no mutations were detected. Thus, the involvement of another gene that could cause BW has been suggested, but no other gene has been identified so far [12]. The most common mutation is missense substitution type, other variants are frameshift, nonsense substitution, and splicing type. In general, the frameshift and missense mutation is associated with a more severe phenotype [2]. Rare mutations are also reported, such as regulatory type, deletion of an entire exon, the presence of 3 concomitant pathogenic variants, and monogenic dysomia [14].

Using guidelines from the American College of Medical Genetics and Genome (ACMG) and the Molecular Pathology Association (AMP), was created a clinically relevant electronic source for WD that includes genetic variants of the ATP7B gene reported through February 2019, manually selected from the literature and 6 official international databases associated with WD. The source is available at http://clingen.igib.res.in/WilsonGen/. Therefore, the database formed includes 3662 genetic variants of ATP7B, which can be found in different places of the gene; of which 1458 proved to be unique. The unique variants were classified according to several principles: the type of mutation (substitution, insertion, deletion, deletion/insertion), the functional consequences (frameshift, Stop loss, Stop gain, Start loss, introns, and splicing site), and according to the ACMG guide (pathogenic variant, probably pathogenic, benign, probably benign, of unknown significance) [15].

Mutations in unique amino acid sequences, located in different areas of the ATP7B protein, cause WD. His1069Q – is the best-known mutation, with an allele frequency in the general population ranging from 10-40%, and in the European population being 30-70% [16]. It occurs in the N-domain SEHPL sequence by substituting histidine → glutamic acid at position 1069 of the ATP7B polypeptide chain, resulting in a misfolding of the protein in this domain, with abnormal phosphorylation in the P-domain and a reduced affinity for ATP, a thermally unstable and aberrantly localized protein, not in the Golgi complex, but the reticulum endoplasmic [10, 12]. Other pathogenic variants of this sequence are: E1064A (affecting the protein by the inability to bind the molecule to ATP), G1099, G1101, and I1102 [17].

Mutation in the DKTGT sequence does not allow the relocation of the protein to the site of biliary excretion of Cu, respectively with its intrahepatic accumulation; mutation in the TGEA sequence – inhibits the recovery of cytosol protein in the Golgi complex, when Cu levels are reduced; CPC mutation – C985T – leads to complete loss of Cu transport activity. The protein segment 37SFAFDNVGYEG45 (located just before the MBD1 – metal-binding domain) interacts with the N domain and directs the ATP7B protein to the apical region of the hepatocytes to ensure biliary excretion of Cu as its concentration increases and the N41S mutation in this segment changes intracellular protein behavior, causing bladder trafficking even when Cu is not high, also affects the targeting of the canalicular surface [18]. Missense mutations in MBD5 and MBD6 are associated with WD, and the change in serine → alanine, an amino acid located between MBD3 and MBD4, induces redistribution of ATP7B protein to peripheral endo/lysosomes [19]. The presence of the mutation in the MBDs linker causes WD; for example, in the MBD1-MBD2 linker – R136W substitution; in the MBD3-MBD4 linker – G333> R mutation; in the MBD4-MBD5 linker – A486S mutation; in the segment connecting MBD6-TM1 – a group of mutations H639Y, L641S, D642H and pMet645Arg (the most common pathogenic variant of missense type in Spain 27%) [7]. Mutations that occur near to N-terminus can lead to a complete blockade of protein synthesis, which is associated with severe WD evolution [18]. LLL mutations associated with WD have not been detected, however, several mutations that cause WD to lead to fragmentation of the C-terminal region containing LLL, and this is likely to affect the recognition of ATP7B by adaptive proteins. Consequently, the mutant ATP7B protein remains peripherally blocked, and its recovery in the trans-Golgi network is suppressed [19]. Missense T1434M or Q1399Rfs mutations in the C-terminal 1450DKWSL1454 sequence are also associated with WD [20].

Patients with WD present with genetic heterogeneity in different races and geographical regions. For example, the H1069Q mutation of ATP7B is more prevalent in patients of European origin, such as those in Italy, Romania, and Sweden, while the R778L mutation is more common in East Asia, but neither of these mutations has been reported in India, where another 17 new mutations have been identified. Theoretically, a full spectrum of mutations would be useful in establishing a pre-symptomatic diagnosis for patients with BW; however, due to the mutational variability and heterozygosity of the ATP7B gene, it is difficult to use mutation as a diagnostic tool [21].

According to the data published by Hlistun V., Sacara V. and colleagues [22], a clinical-molecular evaluation of 120 patients with WD in the Republic of Moldova (40 patients genetically examined in Germany, and 80 – at the Genetic Center of the Republic of Moldova), 18 different mutations were identified, of which 4 new variants, the most common pathogenic variants being p.H1069Q (exon 14) and p.G1314D (exon 20), and in 13 patients evaluated in Germany no mutation was determined.

Epigenetic mechanisms. It is established that the accumulation of Cu in patients with WD is due to mutation in the ATP7B gene encoding the ATP7B protein. However, it is suggested that the presence of other factors, such as epigenetic mechanisms – which act at the interface between the genome and environmental factors and modify genes – could be involved in altering Wilson's gene expression and clinical variety and disease progression. In addition, environmental factors, such as diet, lifestyle, and exposure to toxins, both in utero and postnatal, can affect gene expression through epigenetic mechanisms [23].

Epigenetic mechanisms include reversible changes in DNA or histones, thereby affecting gene expression without altering the DNA sequence. Gene expression is regulated at various levels, including the chromatin remodeling stage, which is composed of DNA, histones, and non-histone proteins. Chromatin remodeling is initiated with DNA methylation, by the addition of methyl groups (H_3C) to DNA from methyl donors –betaine, choline, folate, and methionine. Many methyl donors and other structures involved in the metabolism of methyl donors are essential nutrients, respectively, that must be obtained from the food. DNA methylation requires a sufficient supply of methyl donors, such as methionine, and the changes that underlie its metabolism can affect DNA methylation and, ultimately, disease progression [24].

Major mechanisms involving histone changes, such as acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and ribosylation, indirectly affect gene expression. These are complex cellular mechanisms that induce changes in protein function, chromatin conformation, and gene expression; they can also stimulate protein inactivation/degradation and initiate cellular apoptosis. Another third mechanism is the action of non-coding RNAs. MicroRNAs increase the degradation of messenger RNA required in the process of gene transcription and protein synthesis, thus affecting gene expression by inducing "silence". Long RNAs influence gene expression by interacting with chromatin-modifying proteins, regulating their translation process and stability. However, these mechanisms are to be studied in WD [25].Of particular interest is the interaction between mitochondria and epigenome. Mitochondria are more susceptible to damage by excess Cu compared to other cell compartments, and in patients with WD, their morphology is altered in both early and late stages. It inhibits key enzymes in mitochondria, thus disrupting the production of metabolites derived from mitochondria that are involved in epigenetic regulation. Mitochondria are also a major production site for reactive oxygen species, which in normal concentrations act as signals, but increasing intracellular concentrations can induce oxidation of DNA, lipids, and mitochondrial proteins, degrading mitochondria and causing posttranslational changes (conformation, activity, location) of cellular proteins [23, 25].

Modifying genes are genes that affect the expression of another gene located in another locus or the phenotypic expression of another gene. Such genes are known to influence the evolution of WD. Thus, the role of the genes APOE (apolipoprotein E), COOMD1 (copper metabolism domain-containing 1), ATOX1 (human homolog antioxidant 1 copper chaperone), and HFE (hemochromatosis) has not been confirmed in large studies. [49] Additional research is also needed to demonstrate the involvement of ATPase copper transporting alpha (ATPase) genes, DMT1 (divalent metal transporter 1), PNPLA3 (patatin-like phospholipase domain-containing 3 genes), PRNP (Prion Protein), XIAP (X-linked inhibitor of apoptosis protein), ESD (esterase D), INO80 (INO80 complex subunit) and MTHFR (5, 10-methylenetetrahydrofolate reductase) in the pathogenesis of WD [23].

Epigenetic changes that are caused by environmental factors (e.g., diet, exercise, stress, and toxins) can exacerbate the changes induced by excess Cu, thereby altering the clinical picture and disease progression, and improving these factors provides an opportunity to mitigate the toxic effect of Cu in patients with WD. Kieffer D. and Medici V. [24] proposed the multi-hit hypothesis by which Cu and lifestyle can change the phenotype in WD. In the case of a mother pregnant with a homozygous fetus with an ATP7B mutation, the first hit is caused by exposure to the maternal toxic environment, namely the diet high in fructose, sedentary lifestyle, social stress, and contact with various toxins. The second hit follows after the birth of the child, due to the pathogenic mutation that determines the accumulation of Cu in the organs with the appearance of mitochondrial dysfunction and the alteration of the epigenetic mechanisms of regulation of gene expression. Subsequent hits are caused by exposure to own toxic environmental factors, such as a diet high in fructose and lipids, sedentary lifestyle, social stress, and contact with various toxins. These factors aggravate pre-existing mitochondrial lesions and worsen the epigenetic mechanisms of gene expression regulation. Finally, the disease begins early and/or presents with a severe evolution of WD. However, these suggestions need to be studied and confirmed for their role in WD progression [24].

The close interrelationship between Cu accumulation, methionine metabolism, mitochondrial function, and gene expression regulation, with strong evidence from animal model studies, indicates that epigenetic phenomena most likely contribute to phenotypic diversity in WD, and their analysis is essential for understanding multiple factors interacting in metabolic and complex liver disease – including WD [25].

Microbiome and Wilson's disease. The human intestinal microbiota has a wide range of unique genes encoding different proteins, which are not present in humans, thus greatly expanding the genetic potential of the host; can produce metabolites capable of altering DNA expression, thus exerting epigenetic regulation of human gene expression; changes in various diseases, both liver and neurological; it changes rapidly in response to food and medicine. Lichtmannegger J. and colleagues [25], explored, in animal models, the efficacy of a microbiota-derived peptide,

methanobactin, which has a high affinity for Cu. Short-term treatment with methanobactin regressed mitochondrial and liver damage in treated ATP7B-deficient rats compared to untreated ones [25]. This area of research is largely unexplored but may offer new treatment options, especially for liver damage, as the liver is the first organ to come into contact with metabolites derived from intestinal microorganisms [26].

Gender differences and Wilson's disease. Few studies have investigated the effects of Cu accumulation and sex differences in WD pathogenesis. In a study by Ferenci P. [27], more than 1000 patients with WD, it was reported that sex and age are modifiers of clinical presentation. Acute hepatic impairment occurs more frequently in young women, and the neurological picture predominates in men; while neurological manifestations in children are rare. Data confirmed in previous studies. Litwin T. and colleagues [28], in their research that included 627 patients with WD in Poland, showed that the neuropsychiatric phenotype predominates in men compared to women. Hepatic phenotype occurs more frequently in women, and neurological manifestations develop later than in men. Thus, it has been suggested that the differences are due to the protective effects of estrogen on neurons, which delay the onset of neurological damage, differences in iron metabolism, and occupational factors. However, no conclusive studies have been performed to confirm these claims.

Identification of the phenotype-genotype correlation. WD is characterized by a high mutational variety and an unpredictable clinical picture, and despite the response to treatment and autosomal recessive transmission, the mechanism of phenotypic diversity is currently unknown. Several studies have attempted to find a correlation between genotype and clinical phenotype, but recent studies in a large number of patients have not identified any association [29]. However, some research suggests a possible relationship between the age of onset or type of clinical presentation and a specific genotype [8].

One of the strongest genotype-phenotype correlations in WD is that mutations that cause loss of ATP7B protein function, especially early codon stop mutations (with the synthesis of an abnormally short and unstable protein) and those in functionally important regions, are associated with severe evolution, early-onset, and a predominantly hepatic phenotype, while mutations that partially retain Cu transport function present with a milder evolution. Point mutations in less important regions of the gene are associated with late-onset and a predominant neurological or psychiatric phenotype [12].

Kalita J. and colleagues [30] observed that most patients with the R778L mutation present with a hepatic phenotype and an earlier onset of the disease, while patients with the H1069Q substitution present with a neurological phenotype and a later onset. Kayser-Fleischer rings are more common at the time of diagnosis in H1069Q homozygous patients than in compound heterozygous patients.

A study conducted by Cocos R. and colleagues [8], of 2 large families in the mountainous region of Romania, with 50 members – 7 affected by WD, reported a high intra-familial concordance of patients with WD, with predictability higher for neurological presentation with the same set of clinical features at the time of diagnosis and identical age at the onset of the disease; such data being observed by other researchers. Also, the coexistence of H1069Q and frameshift p.M769H-fs substitution mutations in compound heterozygous patients was associated with a lower age at onset, coinciding with previous reports of the age of onset for homozygous/heterozygous patients with p. H1069Q. A family study by Chabik G. and colleagues [31] found that although the type of clinical presentation varies, siblings present with the same phenotype or age at the onset of the disease, while research by Czlonkowska A. and colleagues [32], did not reveal any genotype-phenotype correlation in homozygous/heterozygous siblings composed of the same mutation or monozygotic twins, the studies suggesting the involvement of genetic and environmental factors that could influence the phenotype.

Mihaylova V. and colleagues [33], in their research including 123 patients with WD from Bulgaria, observed a lack of correlation in patients with p.H1069Q homozygous/heterozygous compound mutation or patients without this mutation with neurological phenotype. The homozygous p.H1069Q mutation was associated with the more frequent hepatic phenotype and the significantly rarer presence of the Kayser-Fleischer ring than with the compound heterozygous p.H1069Q mutation and other mutations. In contrast, patients with the p.R616Q mutation, either homozygous or compound heterozygous, had significantly more frequent neurological manifestations and a higher level of ceruloplasmin than in homozygotes with p.H1069Q and patients without p.H1069Q; in all subjects with the p.R616Q mutation, the Kayser - Fleischer ring was detected.

Usta J. and colleagues [16], evaluated a Lebanese family of 76 members, in which inbreeding is present, in 9 members diagnosed with WD. All 9 patients had the mutation c.2299insC, 5 were homozygous and 4 were heterozygous compounds with p.Ala1003Thr. The study reported a correlation between c.2299insC homozygous patients with liver disease and c.2299insC/p.Ala1003Thr compound heterozygous patients with neurological disease.

The results of a Chinese study by Cheng N. and colleagues [34], which included 1222 patients with WD, showed that the pArg778Leu mutation correlated with an onset at a younger age and with lower levels of ceruloplasmin and serum Cu. Other variants of pArg919Gly and pThr935Met were associated with higher levels of ceruloplasmin, the pArg919Gly variant being correlated with neurological symptoms, while pThr935Met with combined neurological and visceral manifestations. The greater the impact of the mutation on the structure and function of the ATP7B protein, the earlier the onset of the disease and the lower the level of serum ceruloplasmin.

A report by Takeshita Y. and colleagues [35], of 2 unrelated families in Japan, in which family members with WD had similar mutations in the ATP7B gene, found that all members of the same family had different phenotypes and the onset of the disease at different ages. Thus, it was suggested that there may be a difference in allelic dominance (in the identified mutations), as all patients in both families were found to be compound heterozygotes. This was also confirmed by Sapuppo A. and colleagues [36], in the case of 2 sisters with WD, heterozygous compounds with similar genotype (c.3207C-> A (p.H1069Q) / c.3904-2A-> G), which presented with different phenotypes both at the beginning and during the disease, being presumed several factors that would change the evolution of the disease (initiation of therapy in the asymptomatic phase, lifestyle differences, possible intervention of modifying genes of Cu metabolism). Yahata S. and colleagues [37] examined 11 Japanese families, including 23 brothers diagnosed with WD, and reported that in 5 families the disease had the same phenotype, while in the other 6 families with a different phenotype.

Research conducted in India by Santosh S. et al. [38], investigated the impact of 4 different mutations on members of 4 unrelated families (>1 member affected by WD), patients were evaluated to identify correlations between genotype (mutation type, homozygous/heterozygous compound) and phenotype (clinical presentation, age of onset of disease/ age of diagnosis, serum ceruloplasmin level and urinary Cu within 24 h). The results of the research evoked a strong genotype-phenotype concordance between members of the same family, except for 2 minor phenotypic differences between members of 2 families, this being explained by the evolution of the long-term untreated disease, which could be a confusing factor in establishing correlations.

Thus, genetic polymorphism, unique pathogenic variants, atypical mutational mechanisms, the presence of compound heterozygotes, rarity of the disease, clinical variety, epigenetic factors, as well as socio-demographic characteristics (sex, race, and age) complicate the process of associating a genetic variant with a certain phenotype. Efforts to identify some genotype-phenotype interrelationships have shown conflicting results, as the mechanisms of WD heterogeneity are not fully elucidated, especially due to the unpredictable effects of mutations on the structure and functionality of the ATP7B protein [12].

Conclusions

Wilson's disease is a complex disease manifested by mutational heterogeneity and a polymorphic clinical picture. The results of genotype-phenotype correlation studies are not well defined, and in some cases are completely contradictory. The interaction between genetic mutations and epigenetic factors may explain the phenotypic variability. Determining the mechanisms of varied clinical presentation in WD is a challenge for contemporary researchers, and understanding the biochemical, genetic, and physiological processes at the micro-/macromolecular level, as well as at the macro-organism level, could highlight additional avenues in elucidating the genotype-phenotype interrelationship conflict and identify potential therapeutic targets.

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VC conceptualized the idea, conducted a literature review, wrote the manuscript, revised and finalized the text.

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Ethics approval and consent to participate
No approval was required for this study.

Conflict of interests
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