Clinical spectrum and genotype-phenotype associations in Finnish patients with Wilson's disease

Jussi O.T. Sipilä M.D., Ph.D., B.Soc.Sci.[1,2], Laura Kytövuori, Ph.D.[3,4] Valtteri Kaasinen M.D., Ph.D.[1,5]

[1] Clinical Neurosciences, University of Turku, Turku, Finland

[2] Department of Neurology, Siun Sote North Karelia Central Hospital, Joensuu, Finland

[3] Research Unit of Clinical Medicine, Medical Research Center Oulu, Finland

[4] Neurocenter, Neurology, Oulu University Hospital and University of Oulu, Oulu, Finland

[5] Neurocenter, Turku University Hospital, Turku, Finland

Corresponding Author: Jussi Sipilä, M.D., Ph.D., B. Soc. Sci. Division of Clinical Neurosciences, Turku University Hospital, POB 52, FI-20521 Turku, Finland

e-mail: jussi.sipila@utu.fi

Word count: 1405 **Abstract word count**: 150

Tables: 1 **Figures:** 0

Key words: Hepatolenticular Degeneration, Hereditary neurodegenerative diseases, Clinical neurology, Hepatology, Neuroepidemiology, Neurogenetics

Financial disclosure related to research covered in this article: Nothing to declare

Funding: This study was supported by grants from the Finnish Parkinson Foundation, the Finnish Cultural Foundation and by the public VTR-funding of Turku University Hospital. The sponsors had no role in study design, data collection, data analysis, data interpretation or writing of the article. The authors had full and unimpeded access to all data and the final responsibility for the decision to submit for publication.

ABSTRACT

Genotype-phenotype correlation data covering all ages of Wilson's disease onset in Caucasian patients are limited. We therefore analyzed genotype-phenotype correlations in a retrospective cohort of Finnish patients. Six homozygous (HoZ) and 11 compound heterozygous (CoHZ) patients were included. There were no differences in the presence/absence of hepatic, neurological, psychiatric or any symptoms at diagnosis ($p>0.30$ for all) between HoZ and CoHZ patients, but HoZ patients had an earlier age of diagnosis (median 6.7 versus 34.5; $p=0.003$). Severe liver affliction was almost exclusively associated with the p.H1069Q variant. Patients with p.H1069Q had a later mean age of diagnosis (30.2 ± 11.6 vs. 8.7 ± 4.9 years; $p<0.001$) compared to those without. There were no differences in the presence/absence of hepatic, neurological, psychiatric or any symptoms at diagnosis between p.H1069Q-positive and p.H1069Q-negative patients ($p>0.54$ for all). These results suggest that population-specific factors may partly explain the high clinical variability of Wilson's disease.

Introduction

Wilson's disease (WD) is a copper accumulation disorder caused by *ATP7B* mutations that disturb the functioning of the synonymous transporter in hepatocytes and therefore induce a failure of biliary copper excretion.[1,2] Its mode of inheritance is recessive, and the penetrance is unclear, with over 600 mutations identified as probably or possibly pathogenic.[3,4] Disease onset may occur at any time between infancy and the 8th decade of life. The phenotype is variable, with patients typically presenting with either primarily liver or brain affliction but sometimes both. The disease is potentially life-threatening but fortunately usually treatable. Nevertheless, survival rates are usually reported to be lower compared to matched control populations.[5-7]

Prognostic predictors for WD are clearly needed, especially considering the uncertainty about penetrance and the fact that a considerable proportion of patients are diagnosed when asymptomatic. The current criteria allow an established diagnosis of WD based solely on genetic findings.[8] Thus, information on genotype-phenotype correlations is essential because two disease-associated mutations do not inevitably impair copper metabolism raising the risk of clinically unwarranted diagnoses.[9,10]. The current data on the subject are ambiguous.[1] It should, however, be noted that these studies have often been performed in pediatric cohorts,[11-14] and few have only included adult patients.[15] This is particularly important because the hepatic presentation clearly predominates in children.[16] Moreover, much of the data that include patients of all ages are derived from the Far East.[17-20] Considering the well-known differences in the genetic background of WD in different populations,[21,22] especially those between the Chinese and Caucasian populations,[23] it is relevant to investigate genotype-phenotype interactions in patients from different genetic and ethnic backgrounds. In Finland, WD is extremely rare, and up to one-third of patients are immigrants.[6] Moreover, Finns have a peculiar genetic background.[24,25] We therefore investigated the genotype-phenotype correlations of WD in Finnish patients of all onset ages.

Methods

Study design and data source

Epidemiological data and the ascertainment methods of this cohort have been previously described.[6] In brief, all patients treated for WD in 1998-2017 in Finland were identified, diagnoses were validated, and data were obtained using multiple national registries and individual patient records. The diagnosis was accepted if the patient met The European Association for the Study of Liver criteria for Wilson's disease (score 4 or more on the scoring system developed at the 8th International Meeting on Wilson's disease).[8] For the current study, we collected the genotype and phenotype data of these patients from the patient records.

Statistical analysis

Shapiro–Wilk and Kolmogorov–Smirnov tests were used to assess the distribution of continuous variables, and subsequently, Student's t test, the Mann–Whitney U test or independent samples Kruskal–Wallis test were used as appropriate. Crosstabulation and the X^2 test were used to analyze the distribution of categorical variables between groups. P values less than 0.05 were considered significant. IBM SPSS Statistics, Version 26 (IBM SPSS, Chicago, IL, U.S.A.), was used for statistical analyses.

Results

Genotypic data were available for 17 patients, of whom six were homozygotes and 11 were compound heterozygotes (Table 1). There were no differences in the sex distribution between these groups ($p=0.62$) or the presence/absence of hepatic, neurological, psychiatric or any symptoms at diagnosis ($p>0.30$ for all). However, homozygotes had an earlier age of diagnosis (median 6.7, interquartile range 13) than compound heterozygotes (median 34.5, interquartile range 22; $p=0.003$). Serum ceruloplasmin (normal/abnormal) showed no difference between the groups ($p=0.24$).

Liver transplantations were performed only for compound heterozygotes, but this observation was not statistically significant ($p=0.52$). Additionally, one homozygote and one compound heterozygote with a fully known genotype presented with acute liver failure. These two patients and the three patients in whom a liver transplantation had been performed all shared the p.H1069Q mutation. There was only one patient with this mutation who had no severe hepatic manifestations (diagnosed asymptomatic based on genotype and low serum ceruloplasmin at the age of 34 and has remained asymptomatic on penicillamine for over 20 years). Patients with p.H1069Q had a later mean age of diagnosis (30.2 ± 11.6 years) compared to the patients with a known genotype without this mutation (8.7 ± 4.9 years; $p<0.001$). There were no differences in the presence/absence of hepatic, neurological, psychiatric or other symptoms at diagnosis between p.H1069Q-positive and p.H1069Q-negative patients ($p>0.54$ for all). Two brothers with compound heterozygosity but for whom more specific genotypes were unavailable also had cirrhosis of the liver at the time of diagnosis (age 35-39 both). Neither of these patients presented any symptoms (examined because of elevated liver enzymes in donated blood).

Discussion

In this study, we observed that homozygous Finnish patients had been diagnosed with Wilson's disease at a younger age than compound heterozygous Finnish patients. Furthermore, when the genotype included p.H1069Q, the diagnosis was made markedly later, and severe hepatic damage was involved. Our data from a bottleneck population of Finland also provide new insights from a European WD population in which p.H1069Q is not as dominant as it is in many other populations.[26,27]

The majority of previous studies in Caucasian populations have reported that genotype is not associated with the type of initial WD presentation.[28-35] This was our finding as well. Previous data have also consistently reported that patients with two 'severe' (truncating nonsense or frameshift) mutations are associated with an earlier age of disease onset compared to patients with two missense mutations (such as p.H1069Q).[28,33] Interestingly, our data included only one patient who was homozygous for a severe mutation (c.1639delC/Gln547Argfs*22), and his age at diagnosis was similar to those of almost all patients who were homozygotes for missense mutations. This might be a chance occurrence related to the small sample size, but unidentified population-specific modifying factors might also be at play. The same considerations should be applied to our finding that WD was diagnosed much earlier in homozygotes than in compound heterozygotes. However, previous studies have not specifically analyzed homozygosity for specific mutations, only for the state of mutation severity.[28,33,34] This may have been due to the predominance of the p.H1069Q mutation in these data, whereas in our data, the p.H1069Q mutation was not the most common mutation (27% of all identified pathogenic variants).

The importance of the p.H1069Q variant for the overall analysis is highlighted by the fact that the only patient homozygous for this mutation in the cohort was considerably older at the time of diagnosis compared to the other homozygous patients. This is further compounded by the finding that the presence of p.H1069Q was associated with a later age at diagnosis in all patients. Previous

data from Greece and the Netherlands, along with a meta-analysis, have associated the p.H1069Q variant with a later age of WD onset, whereas studies conducted in Serbia, the Czech Republic and Slovakia reported no such correlation.[29,31,34,35] This discrepancy may result from population-specific factors or differences in analytical methods. The fact that the p.H1069Q variant has been associated with both a predominantly neurological and a predominantly hepatic phenotype underscores this.[35,36] Our results that show that p.H1069Q is associated with severe liver damage in Finnish patients, which is rather similar to the results reported from Lithuania.[36] These findings suggest that region- and/or population-specific factors might be important. The nature of Finland as a genetic isolate may therefore limit the generalizability of these results, especially considering that WD is exceptionally rare in Finland.[6,22,24,25] However, these populations may offer unique insights, [26,27] as also shown by our results concerning homozygosity. These also include the observation that all patients who were homozygous for p.Asp1296Asn had normal urinary copper excretion.

This was a retrospective study relying on registry data and patient charts. The capture-recapture analysis in our previous study suggested excellent case ascertainment.[6] However, since genotype data were available for only 52% of patients and partially lacked specific variant data, the results should be interpreted cautiously. Further and larger studies on this subject are warranted in Finland, and considering that many genotype-phenotype studies were conducted before the current genetic tools became available, timely data are needed from other populations. Moreover, it is evident that the phenotype is modified by multiple genetic ad epigenetic factors,[37] which we were unable to investigate in these data.

In conclusion, in Finnish WD patients, the age at diagnosis was younger in homozygotes than compound heterozygotes, and the p.H1069Q variant was associated with a higher age at diagnosis. Additionally, almost all p.H1069Q variant patients had severe liver damage. These findings, along

with previous findings from other regions, suggest that there are also population-specific factors that explain the high clinical variability of WD.

Data availability

This manuscript is based on third-party data. Access to data is regulated by Finnish law and the Finnish National Institute for Health and Welfare. The disclosure of data to third parties without explicit permission from the Finnish National Institute for Health and Welfare is prohibited. Only those fulfilling the requirements established by Finnish law and the Finnish National Institute for Health and Welfare for viewing confidential data can access the data. We confirm that the authors did not have any special access privileges that others would not have.

Ethics

This registry-based study was approved by the Finnish National Institute for Health and Welfare (1863/5.05.00/2017) and Statistics Finland (TK-52-1763-19). In addition, regional permits were required by some hospital districts, and they were obtained accordingly. Since the study involved no contact with patients, ethics committee approval was unnecessary. This was a retrospective register study. Thus, no informed consent was needed, and the participants were not contacted. The legal basis for processing personal data is public interest and scientific research (EU General Data Protection Regulation 2016/679 (GDPR), Article 6(1)(e) and Article 9(2)(j); Data Protection Act, Sections 4 and 6).

Author contributions

Jussi Sipilä and Valtteri Kaasinen designed and supervised the study and gathered the clinical and genetic data. All authors analyzed the data. Jussi Sipilä wrote the article draft. Laura Kytövuori and Valtteri Kaasinen revised the manuscript.

REFERENCES

1. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. Nat Rev Dis Primers. 2018; 4: 21.
2. Coffey AJ, Durkie M, Hague S, et al. A genetic study of Wilson's disease in the United Kingdom. Brain. 2013; 13: 1476–1487.
3. Kumar M, Gaharwar U, Paul S, et al. WilsonGen a comprehensive clinically annotated genomic variant resource for Wilson's Disease. Sci Rep. 2020; 10: 9037.
4. Wallace DF, Dooley JS. ATP7B variant penetrance explains differences between genetic and clinical prevalence estimates for Wilson disease. Hum Genet. 2020. 139: 1065-1075.
5. Beinhardt S, Leiss W, Stättermayer AF, et al. Long-term outcomes of patients with Wilson disease in a large Austrian cohort. Clin Gastroenterol Hepatol. 2014; 12: 683-689.
6. Sipilä JOT, Hietala M, Kytö V, Kaasinen V. Wilson's Disease in Finland: A Nationwide Population-Based Study. Mov Disord. 2020; 35(12): 2323-2327
7. Bruha R, Marecek Z, Pospisilova L, et al. Long-term follow-up of Wilson disease: natural history, treatment, mutations analysis and phenotypic correlation. Liver Int. 2011; 31(1): 83-91.
8. European Association for Study of Liver. EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol. 2012; 56: 671-685.
9. Stättermayer AF, Entenmann A, Gschwantler M, et al. The dilemma to diagnose Wilson disease by genetic testing alone. Eur J Clin Invest. 2019; 49: e13147.
10. Antos A, Litwin T, Skowrońska M, Kurkowska-Jastrzębska I, Członkowska A. Pitfalls in diagnosing Wilson's Disease by genetic testing alone: the case of a 47-year-old woman with two pathogenic variants of the ATP7B gene. Neurol Neurochir Pol. 2020; 54(5): 478-480.
11. Nicastro E, Loudianos G, Zancan L, et al. Genotype-phenotype correlation in Italian children with Wilson's disease. J Hepatol. 2009; 50(3): 555-61.

12. Pop TL, Grama A, Stefanescu AC, et al. Acute liver failure with hemolytic anemia in children with Wilson's disease: Genotype-phenotype correlations? World J Hepatol. 2021; 13(10): 1428-1438.
13. Abdel Ghaffar TY, Elsayed SM, Elnaghy et al. Phenotypic and genetic characterization of a cohort of pediatric Wilson disease patients. BMC Pediatr. 2011; 11: 56.
14. Zhu Q, Zhu K, Wang J, et al. Relationship between genetic mutations and clinical phenotypes in patients with Wilson disease. Medicine (Baltimore). 2019; 98(49): e18284.
15. Vieira Barbosa J, Fraga M, Saldarriaga J, et al. Hepatic manifestations of Wilson's disease: 12-year experience in a Swiss tertiary referral centre. Swiss Med Wkly. 2018; 148: w14699.
16. Fernando M, van Mourik I, Wassmer E, et al. Wilson disease in children and adolescents. Arch Dis Child 2020; 105: 499–505.
17. Okada T, Shiono Y, Kaneko Y, et al. High prevalence of fulminant hepatic failure among patients with mutant alleles for truncation of ATP7B in Wilson's disease. Scandinavian Journal of Gastroenterology 2010; 45(10): 1232-7.
18. Okada T, Shiono Y, Hayashi H, et al. Mutational analysis of ATP7B and genotype-phenotype correlation in Japanese with Wilson's disease. Hum Mutat. 2000; 15(5): 454-62.
19. Cheng N, Wang H, Wu W, et al. Spectrum of ATP7B mutations and genotype-phenotype correlation in large-scale Chinese patients with Wilson Disease. Clin Genet. 2017; 92(1): 69-79.
20. Lee BH, Kim JH, Lee SY, et al. Distinct clinical courses according to presenting phenotypes and their correlations to ATP7B mutations in a large Wilson's disease cohort. Liver Int. 2011; 31(6): 831-9.
21. Ferenci P. Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. Hum Genet. 2006; 120(2): 151-9.

22. Gao J, Brackley S, Mann JP. The global prevalence of Wilson disease from next-generation sequencing data. Genet Med. 2019; 21(5): 1155-1163.
23. Wu ZY, Wang N, Lin MT, et al. Mutation analysis and the correlation between genotype and phenotype of Arg778Leu mutation in chinese patients with Wilson disease. Arch Neurol. 2001; 58(6): 971-6.
24. Cavalli-Sforza LL, Piazza A. Human genomic diversity in Europe: a summary of recent research and prospects for the future. Eur J Hum Genet. 1993; 1: 3-18
25. Lao O, Lu TT, Nothnagel M, et al. Correlation between Genetic and Geographic Structure in Europe. Curr Biol. 2008; 18(16): 1241-8.
26. Chheda H, Palta P, Pirinen M, et al. Whole-genome view of the consequences of a population bottleneck using 2926 genome sequences from Finland and United Kingdom. Eur J Hum Genet. 2017; 25(4): 477-484.
27. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023; 613(7944): 508-518.
28. Merle U, Weiss KH, Eisenbach C, et al. Truncating mutations in the Wilson disease gene ATP7B are associated with very low serum ceruloplasmin oxidase activity and an early onset of Wilson disease. BMC Gastroenterol. 2010; 10: 8.
29. Tomić A, Dobricić V, Novaković I, et al. Mutational analysis of ATP7B gene and the genotype-phenotype correlation in patients with Wilson's disease in Serbia. Vojnosanit Pregl. 2013; 70(5): 457-62.
30. Sapuppo A, Pavone P, Praticò AD, et al. Genotype-phenotype variable correlation in Wilson disease: clinical history of two sisters with the similar genotype. BMC Med Genet. 2020; 21(1): 128.

31. Vrabelova S, Letocha O, Borsky M, Kozak L. Mutation analysis of the ATP7B gene and genotype/phenotype correlation in 227 patients with Wilson disease. Mol Genet Metab. 2005; 86(1-2): 277-85.
32. Møller LB, Horn N, Jeppesen TD, et al. Clinical presentation and mutations in Danish patients with Wilson disease. Eur J Hum Genet. 2011; 19(9): 935-41.
33. Gromadzka G, Schmidt HH, Genschel J, et al. Frameshift and nonsense mutations in the gene for ATPase7B are associated with severe impairment of copper metabolism and with an early clinical manifestation of Wilson's disease. Clin Genet. 2005; 68(6): 524-32.
34. Panagiotakaki E, Tzetis M, Manolaki N, et al. Genotype–Phenotype Correlations for a Wide Spectrum of Mutations in the Wilson Disease Gene (ATP7B). Am J Med Genet A. 2004; 131(2): 168-73.
35. Stapelbroek JM, Bollen CW, van Amstel JK, et al. The H1069Q mutation in ATP7B is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. J Hepatol. 2004; 41(5): 758-63.
36. Kucinskas L, Jeroch J, Vitkauskiene A, et al. High frequency of the c.3207C>A (p.H1069Q) mutation in ATP7B gene of Lithuanian patients with hepatic presentation of Wilson's disease. World J Gastroenterol. 2008; 14(38): 5876-9.
37. Kluska A, Kulecka M, Litwin T, et al. Whole-exome sequencing identifies novel pathogenic variants across the ATP7B gene and some modifiers of Wilson's disease phenotype. Liver Int. 2019; (39) :177-186.