Research article

JHEP|Reports

HSD17B13 truncated variant is associated with a mild hepatic phenotype in Wilson's Disease

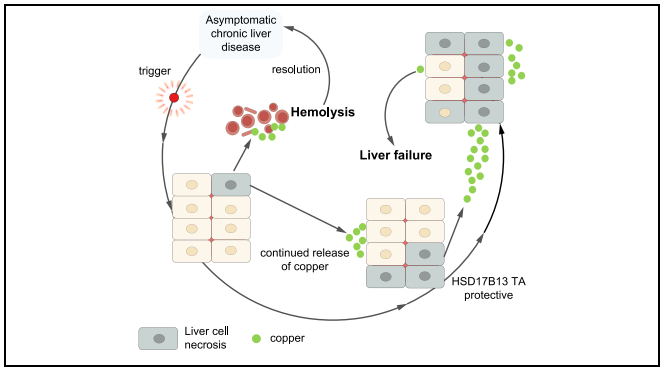
Authors

Peter Ferenci, Jan Pfeiffenberger, Albert Friedrich Stättermayer, Rudolf E. Stauber, Claudia Willheim, Karl H. Weiss, Petra Munda-Steindl, Michael Trauner, Michael Schilsky, Heinz Zoller

Correspondence

peter.ferenci@meduniwien.ac.at (P. Ferenci)

Graphical abstract



Highlights

- Wilson's disease is a hereditary liver disease caused by impaired biliary copper excretion
- A gene mutation (*HSD17B13*:TA) modifies the degree of liver pathology
- Patients carrying this mutation more frequently have milder liver disease
- In most patients with fulminant Wilson's disease this mutation is absent
- *HSD17B13*:TA appears to offer some degree of protection against copper toxicity

Lay summary

Wilson's disease is a hereditary disease caused by accumulation of copper in the liver and other tissues. It presents with a variety of clinical symptoms. In this study we explored the role of a recently described gene mutation (HSD17B13:TA) which apparently protects the liver against toxins like alcohol. The results indicate that this mutation plays a role in the evolution of liver disease. Patients with Wilson's disease who carry this mutation are more likely to have mild disease, while the absence of the mutation is associated with the most severe form – fulminant Wilson's disease.

https://doi.org/10.1016/j.jhepr.2019.02.007

HSD17B13 truncated variant is associated with a mild hepatic phenotype in Wilson's Disease

Peter Ferenci,[1,*] Jan Pfeiffenberger,[2] Albert Friedrich Stättermayer,[1] Rudolf E. Stauber,[3] Claudia Willheim,[1] Karl H. Weiss,[2] Petra Munda-Steindl,[1] Michael Trauner,[1] Michael Schilsky,[4] Heinz Zoller[5]

[1]*Internal Medicine III, Dvision of Gastroenterology and Hepatology, Medical University of Vienna, Austria;* [2]*Internal Medicine IV, Medical University of Heidelberg, Germany;* [3]*Internal Medicine, Medical University of Graz, Austria;* [4]*Departments of Medicine and Surgery, Division of Digestive Diseases and Immunology and Transplant, Yale University, New Haven, CT, USA;* [5]*Medical University of Innsbruck, Austria*

JHEP Reports **2019.** https://doi.org/10.1016/j.jhepr.2019.02.007

Background & Aims: *HSD17B13* encodes hydroxysteroid 17-β dehydrogenase 13, a novel liver lipid-droplet associated protein that is involved in the regulation of lipid biosynthetic processes. A protein-truncating *HSD17B13* variant (rs72613567) was shown to protect individuals from alcoholic and non-alcoholic liver disease. Since steatosis is a common feature in Wilson's disease (WD), we aimed to assess whether the HSD17B13 variant modulates the phenotypic presentation and progression of WD.

Methods: The *HSD17B13*:TA (rs72613567) variant was determined by allelic discrimination real-time PCR in 586 patients. The *HSD17B13* genotype was correlated with the phenotypic presentation. The age of onset and the type of symptoms at presentation were used as markers of the WD phenotype.

Results: The overall *HSD17B13*:TA allele frequency in patients with WD was 23.3% (273/1,172), not significantly different from the reported minor allele frequency. There was a significantly lower *HSD17B13*:TA allele frequency in patients with fulminant WD compared to all other phenotypic WD groups (11.0% *vs.* 24.0%, $p < 0.01$). Among the patients with fulminant WD there was a trend for a gender effect; none of the male patients carried the *HSD17B13*:TA allele. *HSD17B13*:TA allele frequency was more common in patients with minimal or no fibrosis (49 [31.1%] had simple steatosis and 20 minimal changes at biopsy) than in patients with cirrhosis or advanced fibrosis (22.3%, $p = 0.025$).

Conclusions: The *HSD17B13*:TA allele modulates the phenotype and outcome of WD. This allele likely ameliorates hepatic fibrosis and reduces the transition from copper induced hemolysis to fulminant disease in patients with WD.

Introduction

Recently, a protein-truncating *HSD17B13* variant (rs72613567) was shown to protect individuals from alcoholic and non-alcoholic liver disease.[1] The association of the T>TA variant with non-alcoholic steatohepatitis and liver fibrosis has been confirmed by independent studies.[2,3] *HSD17B13* encodes hydroxysteroid 17-β dehydrogenase 13, a protein involved in the regulation of lipid biosynthetic processes and that has enzymatic activity for several bioactive lipid species implicated in lipid-mediated inflammation. HSD17B13, an isoform of 17 beta-hydroxysteroid dehydrogenase is highly expressed in the testis and in the liver.[4] Rare variant single nucleotide polymorphisms in *HSD17B13* also affected the lipid lowering effects of fenofibrate.[5]

The novel HSD17B13 isoform encoded by the protective allele is catalytically defective against estradiol.[1] Molecular analysis and recent proteomic studies identified HSD17B13 as a novel liver lipid-droplet associated protein.[6] Recent functional studies demonstrate that the rs72613567 is indeed a loss-of-function variant, since an adenine (A) insertion in the coding gene region (chr4:87310241, GRCh38.p7) adjacent to the donor splice site of exon 6 results in a frame-shift causing premature truncation of the HSD17B13 protein.

These observations may also be relevant in Wilson's disease (WD). The variable phenotypic presentation of WD (for extensive discussion see 7) cannot be explained by the type of *ATP7B* mutation alone.[7] The factors independent of copper accumulation leading to disease progression and to cirrhosis development are unknown. Since hepatic steatosis is also a common feature in WD,[8] we explored whether the HSD17B13 variant might play a role in the phenotypic presentation and progression of WD.

Patients and methods

DNA was available from 585 index patients included in the published phenotype-genotype database[7] (for demographics see Table 1). Referring physicians provided information regarding the patient's history, the time of onset of symptoms (hepatic, neurologic; presence of Kayser–Fleischer rings), the date of diagnosis, and laboratory findings (serum ceruloplasmin, 24 h urinary copper excretion, liver biopsy (n = 404) and hepatic copper content [available in 222]). Diagnosis of WD was based on the Leipzig score.[9,10] The *ATP7B* genotype was determined as described

Received 25 January 2019; received in revised form 31 January 2019; accepted 2 February 2019; available online 19 March 2019

* Corresponding author. Address: Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria.
E-mail address: peter.ferenci@meduniwien.ac.at (P. Ferenci).

previously. Only data of patients with a confirmed diagnosis of WD by Leipzig score ≥4 were used for analysis. The degree of fibrosis (F0-F4) and steatosis was calculated from the description of the local pathologist.

Patients signed an informed consent according to local law requirements. All data (including clinical, demographic and test results) were coded and deposited in the master database. The internal review board of the Medical University of Vienna approved this retrospective analysis (#1372/17).

Definition of phenotype

The age of onset and the type of symptoms at presentation were used as markers of WD phenotype. In patients with both hepatic and neurologic symptoms of disease, the first symptom observed was used to define the phenotype, even if the diagnosis was made much later when other problems were present. Hepatic presentation was subcategorized as fulminant (acute) WD with coagulopathy and hepatic encephalopathy with or without Coombs negative hemolytic anemia, as an independent presentation of Coombs negative hemolytic anemia (occurring in a previously asymptomatic individual without fulminant liver failure and which resolved spontaneously or following treatment with chelation therapy), as decompensated cirrhosis (if it occurred in a patient with known preexisting liver disease) while the remaining patients were classified as having compensated chronic liver disease without Coombs negative hemolytic anemia.

HSD17B13 genotyping assay

The *HSD17B13*:TA (rs72613567) variant was determined by allelic discrimination real-time PCR. The assay was validated by Sanger sequencing in 30 normal controls with different *HSD17B13* genotypes.

Statistics

Statistical analyses were performed with commercially available software (SigmaPlot 13, Systat Software, San Jose, CA, USA). Categorical variables are given as absolute (n) and relative frequencies (%). For comparison of continuous variables, the Student's *t* test and the non-parametric Mann-Whitney *U* test was used, as appropriate. Comparison of categorical variables was performed with Fisher's exact test.

Results

The demographic characteristics of the investigated patients are summarized in Table 1. The overall *HSD17B13:*TA allele frequency in the patients with WD was 23.5%, comparable to published data in Caucasians.[1]

Fulminant WD

Regarding the phenotypic presentation, there was a significantly lower *HSD17B13:*TA allele frequency in patients with fulminant WD compared to all other groups (see Fig. 1). Among the patients with fulminant WD there was a trend for a gender effect. While none of the male patients carried the *HSD17B13:*TA allele, 7 of the 33 female patients were *HSD17B13:*TA heterozygous and one was even a homozygote (allele frequency: 13.6%, $p = 0.07$). Eighteen of the 26 patients with hemolysis underwent liver biopsy and 12 had cirrhosis (66.6%). As expected, 38 of the 41 patients with fulminant WD underwent high urgency liver transplantation and 3 died before a graft was available. A total of 35 of the transplanted patients had cirrhosis (92.3%) and 3 advanced fibrosis. Most of the patients who developed fulminant WD had associated Coombs negative hemolysis, but had more advanced liver disease than those not needing transplantation.

Table 1. Demographics.

		***HSD17B13* genotype**		
	All	**T/T**	**TA/T**	**TA/TA**
N	586	348 (59.4%)	203 (34.6%)	35 (6.0%)
Male/female	302/284	176/171	103/100	22/13
Mean age at onset (yr)	19.6	19.1	20.1	21.3
Presentation				
Fulminant WD	42 (7%)	33 (80.5%)	8 (17.1%)	1 (2.4%)
Hemolysis	26 (4.5%)	16 (61.5%)	8 (30.7%)	2 (7.7%)
Non-cirrhotic + compensated cirrhosis	306 (52.4%)	166 (53.9%)	122 (39.9%)	19 (6.2%)
Decompensated cirrhosis	27 (4.6%)	16 (59.3%)	9 (33.3%)	2 (16.7%)
Neurologic	184 (31.5%)	117 (63.6%)	56 (30.4)	11 (6.0%)
Liver histology available	404			
Cirrhosis	214	130	70	14
Fibrosis (F2/F3) ± steatosis	76	49	23	4
Chronic hepatitis*	45	29	13	3
Steatosis ± mild fibrosis	49	21	26	2
F0, with minimal or no changes	20	9	9	2
ATP7B mutation				
H1069Q/H1069Q	156	102	45	9
H1069Q/other	159	93	56	10
H1069Q/?	59	35	22	2
Other homozygote	57	34	17	6
Other compound heterozygote	95	52	39	4
Other/?	60	33	23	4

* Chronic hepatitis like picture, none had signs of advanced fibrosis or fat accumulation. WD, Wilson's disease.

Fibrosis

*HSD17B13:*TA allele frequency (31.1%) was more common in patients with fibrosis staging 0-1 (49 had simple steatosis and 20 minimal changes at biopsy) than in patients with cirrhosis or advanced fibrosis (22.3%, $p = 0.025$) (Fig. 2), but was not different regarding the age at presentation and the *ATP7B* mutation.

By univariate and multivariate logistic regression analysis only female sex and cirrhosis were significantly associated with severe hepatic disease (Table 2). For this calculation severe hepatic disease was defined as the sum of patients presenting with decompensated cirrhosis, fulminant WD and hemolysis.

Discussion

The major finding in this study was that the *HSD17B13:*TA allele had a potentially protective effect regarding the evolution of fulminant WD. A second result was that patients with mild liver disease (steatosis ± mild fibrosis or minimal changes) had a significantly higher *HSD17B13:*TA allele frequency than patients with more advanced liver disease. These latter findings are in line with reported observations in patients with fatty liver disease.[1–3] The protective effect of *HSD17B13:*TA was primarily seen in patients in the pediatric age group (see Table 2)

Fulminant WD is defined by the acute onset of coagulopathy and hepatic encephalopathy in a patient without evidence of pre-existing liver disease. Jaundice may be present in these patients and may be enhanced by the accompanying Coombs negative hemolytic anemia or due to rapidly deteriorating liver function associated with hepatic decompensation, or a combination of both. The Nazer score/Kings College criteria are useful prognostic scores, but since laboratory data was not available on many of these patients at the time of their illness, we cannot calculate them. Retrospectively, we can assume that those patients with WD who were transplanted and presented with an acute onset of jaundice and liver failure met the definition for fulminant WD. All patients with fulminant WD were transplanted in Austria, Germany, Hungary and Croatia, all members of EUROTRANSPLANT and thus followed the same regulations.

Despite the picture of acute illness with rapid deterioration of liver function, the livers of patients with acute fulminant WD are cirrhotic or at least have advanced fibrosis at the time of clinical presentation. Similarly, most of the patients with Coombs negative hemolysis have cirrhosis. Eighteen of our 26 patients with hemolysis underwent liver biopsy, and 12 had cirrhosis (66.6%), 4 advanced fibrosis, and each one steatosis and minimal changes. As expected, 36 of the 41 patients with fulminant WD underwent high urgency liver transplantation and 5 died before a graft was available. Of the transplanted patients, 33 had cirrhosis (91.6%) and 3 advanced fibrosis. Most of the patients who developed acute fulminant WD started with hemolysis. At onset of jaundice a clear distinction of fulminant WD from WD with episodic hemolysis without liver failure is not possible. Many patients who developed acute fulminant WD had hemolysis prior to presenting with hepatic decompensation.[11]

There are no large series on patients with WD presenting with hemolysis. Most reported cases are case reports. The largest cohort (22 patients out of 321 patients seen) was described by J. Walsh.[12] Most patients were seen before liver transplantation was available. Eight of the 22 patients died shortly after admission of liver failure. Six patients were stable on chelation therapy after 4 to 48 years of follow-up, 8 were lost to follow-up. Roche-Sicot reported 3 cases starting with hemolysis that went on to fulminant liver failure.[13] Two-thirds of the patients with hemolysis in our series had cirrhosis. Meanwhile, many patients who developed fulminant WD had hemolysis at the onset of jaundice.

We defined Coombs negative hemolytic anemia as a distinct entity if it resolved spontaneously or by initiation of chelation therapy. Unfortunately, we did not collect all data necessary to calculate the modified Nazer score[14] in all patients. In an older paper from Germany 8 patients with fulminant WD are shown,[15] 3 of them underwent orthotopic liver transplants (including 2 patients in the current study; Nazer score: 7 and 8; HE grade 1-2). However, selected patients with a high Nazer score may survive due to improvement on medical treatment, as was shown in cases where no graft was available within the first days.[16] Nevertheless, acute liver failure seems to be associated with hemolysis, but hemolysis may also occur in the absence of fulminant hepatic failure.

The mechanisms of copper toxicity are incompletely understood, but copper can initiate free radical generation with subsequent oxidative changes in lipids or thiol proteins within

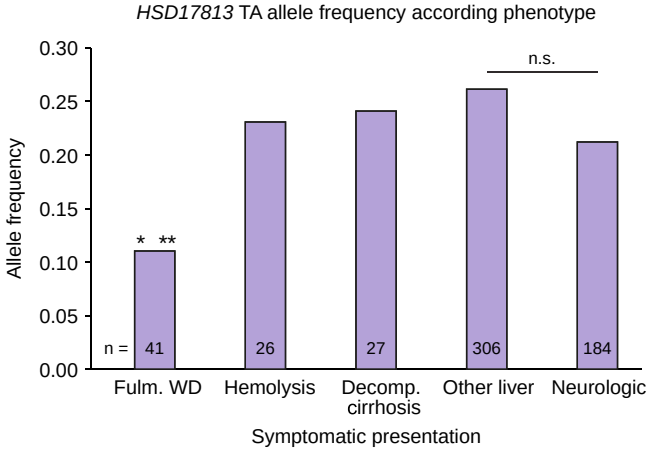


Fig. 1. *HSD13B17*:TA allele frequency according to phenotypic presentation in 584 patients with Wilson's disease. $^{**}p < 0.001$ *vs.* non-fulminant liver disease, $^{*}p < 0.05$ *vs.* neurologic presentation

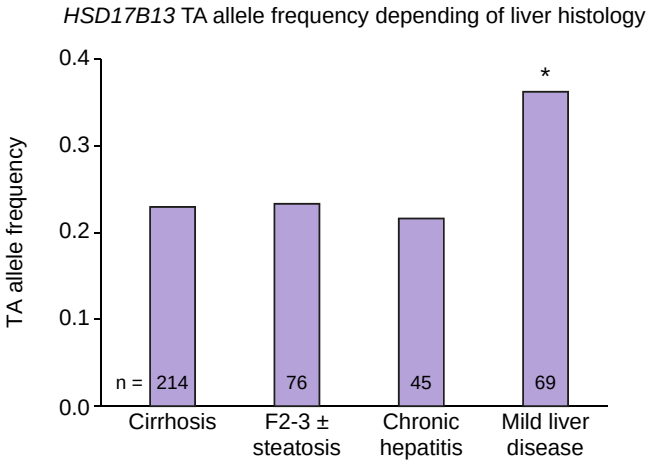


Fig. 2. *HSD13B17*:TA allele frequency according to liver histology at diagnosis in 404 patients with Wilson's disease. Patients with F2-F3 were combined as advanced liver disease (25 has also marked steatosis), those with F0-1 as mild liver disease (20 were F0 with minimal steatosis and 49 with marked steatosis with F0-F1). CH= chronic hepatitis like picture, none had signs of advanced fibrosis or fat accumulation. $^{*}p = 0.02$ *vs.* advanced fibrosis/cirrhosis

Table 2. Univariate and multivariate logistic regression of factors associated with severe hepatic disease.

	Unadjusted			Adjusted		
	OR	95% CI	*p* value	OR	95% CI	*p* value
All patients						
Age (cont.)	1.003	0.982–1.023	0.805			
Sex (male)	0.356	0.222–0.571	<0.001	0.467	0.269–0.811	0.007
Hepatic copper content	1.000	0.999–1.001	0.555			
Cirrhosis	7.147	3.639–14.038	<0.001	6.949	3.512–13.749	<0.001
HSD17B13:TA[*]	0.607	0.378–0.974	0.039	0.512	0.287–0.916	0.024
Truncating mutation[**]	0.880	0.519–1.490	0.633			
Patients <18 years						
Sex (male)	0.268	0.132–0.544	<0.001	0.251	0.105–0.601	0.002
Hepatic copper content (cont.)	1.000	0.999–1.001	0.568			
Cirrhosis	5.035	2.141–11.843	<0.001	4.992	2.047–12.176	<0.001
HSD17B13[*]	0.386	0.183–0.816	0.013	0.381	0.148–0.984	0.046
Truncating mutation[**]	0.996	0.501–1.983	0.992			
Patients >18 years						
Sex (male)	0.463	0.244–0.880	0.019	0.756	0.364–1.571	0.453
Hepatic copper content	0.999	0.998–1.000	0.232			
Cirrhosis	13.147	3.917–44.124	<0.001	12.879	3.833–43.280	<0.001
HSD17B13[*]	0.882	0.469–1.656	0.695			
Truncating mutation[**]	0.681	0.289–1.603	0.379			

Severe hepatic disease was defined as the sum of patients presenting with decompensated cirrhosis, fulminant Wilson's disease and hemolysis.
Liver biopsy was available in 404 patients; Hepatic copper content was measured in 222 patients.
OR, odds ratio.
[*] TA/T or TA/TA.
[**] presence of at least one truncating mutation in ATP7B (N=32; all compound heterozygotes with H1069Q; 2302dupC, 3402delC, W779X, V845Sfs, I945T-fs, G1011X, I1330I-fs, V1282L-fs, Q1372X, V1366G-fs).

hepatocyte organelles.[17] Oxidative stress plays a major role as a common mediator of apoptotic cell death, and cellular copper also causes a reduction in XIAP, further promoting apoptosis. The mechanism of cell death induced by copper likely involves both apoptosis and/or necrosis. Low-dose exposure of various stimulations such as heat, radiation, toxins, hypoxia, and anticancer drugs can induce apoptosis, and the same stimuli at higher dose may result in necrosis.[18]

The following cascade of events may take place: some injury triggers release of copper from hepatocytes. Free Cu is toxic and its sudden increase induces intravascular hemolysis and augments oxidative stress (possibly by release of heme iron that is taken back up into liver cells promoting further oxidative injury) resulting in damage of cellular macromolecules including DNA and proteins, accumulation of wild-type p53 and transcriptional transactivation of CD95L.[19] If copper release continues due to hepatocellular necrosis and apoptosis a vicious chain reaction leads to further progression and ultimately to liver failure. Depletion of glutathione (GSH) and total antioxidant capacity, as well as an increase in reactive oxygen species, malondialdehyde, and cytokines have been documented.[20–22] Furthermore, antioxidants may be protective against induction of CD95L.[19] In line, reduction of oxidative stress has already been incorporated into therapeutic concepts for treatment of fulminant hepatic failure.[23]

HSD13B17 could be involved in several steps of this cascade of events (Fig. 3). Whether the presence of the *HSD13B17*:TA allele is protective or the lower activity/ or absence of the wild-type allele decreases hepatocellular damage is unknown. This protein is involved in the regulation of lipid biosynthetic processes and has enzymatic activity for several bioactive lipid species

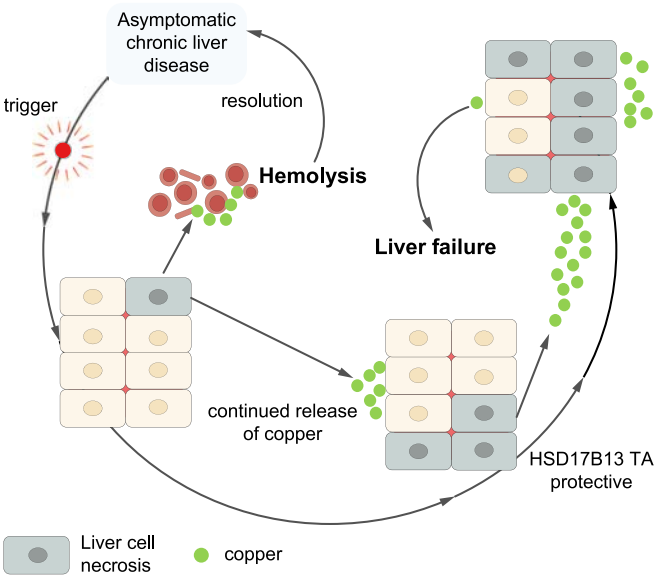


Fig. 3. Schematic presentation of the potential action of HSD17B13 on the evolution of fulminant Wilson's disease. A primary trigger for liver injury leads to hepatocellular necrosis resulting in the release of copper. The increase of copper concentration in serum leads to destruction of erythrocytes, and together with copper in in the extracellular liver tissue to further death of hepatocytes. This cycle of events may ultimately lead to liver failure. We assume that *HSD17B23*:TA may protect hepatocytes.

implicated in lipid-mediated inflammation and retinoid homeostasis.[2] The *HSD13B17*:TA allele leads to a loss-of-function protein. Further studies on the regulation of HSD17B13 expression in the liver of patients with WD are needed to better understand the role of this protein. Since we have not stored liver issues in this study cohort, this must be done in future studies. There is evidence that vitamin A metabolites (retinaldehyde and retinoic acid) and retinol binding protein are associated with pathogenesis of hepatic steatosis, fibrosis, adipogenesis, and insulin resistance.[24,25] The retinol-dehydrogenase activity of HSD17B13 may be involved in the complex nuclear receptor interaction in non-alcoholic fatty liver disease, via activation of the retinoic acid receptor.[26]

An interesting observation is that none of the male patients with fulminant WD carried the *HSD13B17*:TA allele, while 7 of the female patients were TA heterozygotes and one was even *HSD13B17*:TA homozygous ($p = 0.07$). Since the enzymatic activity HSD17B13-isoforms were associated with reduced affinity against estradiol1 this observation further underlines the impact of gender on the phenotypic presentation of WD.[7] The interaction of HSD17B13 with estrogens remains to be investigated.

Limitations of the study are the retrospective data analysis and the lack of a central pathologist. The degree of fibrosis and steatosis was calculated from the description of the local pathologist. This may have impacted the analysis of the *HSD13B17*:TA allele frequency according to liver histology (Fig. 2). Liver biopsies were not performed in all patients. Finally, there was no external validation cohort which was not available due to the rarity of the disease with its highly variable phenotypic expression.

In summary, the *HSD17B13*:TA allele may modulate the phenotype and outcome of WD by reducing the transition from copper induced hemolysis to fulminant WD. Furthermore, it is associated with milder histological changes. Whether these observations can lead to new treatment strategies remains to be explored. However, it may help us better understand the phenotypic variability of WD with respect to specific extragenic effects on the natural history of the disease.

Conflicts of interest

PF: Adboard: Univar, Alexion (former Wilson Therapeutics), Vivet Therapeutics, Gilead, Abbvie, MSD, NovoNordisk; unrestricted research grant: Gilead. KHW: is on the speakers bureaus of AbbVie, Alexion Pharmaceuticals, Bayer, Bristol-Myers Squibb, Chiesi Farmaceutici SpA, GMP-Orphan SAS, Norgine, Novartis, Univar, Wilson Therapeutics and Vivet Therapeutics and has received grants (to the institution) from Alexion Pharmaceuticals, Bayer, Bristol-Myers Squibb, Eisai, GMP-Orphan SAS, Novartis, Univar and Wilson Therapeutics. R.E.S: Speaking and/or consulting fees: AbbVie, BMS, Falk, Gilead, Intercept, Merck/MSD. M.T.: Speaker for BMS, Falk Foundation, Gilead and MSD; advisory boards for Albireo, Falk Pharma GmbH, Genfit, Gilead, Intercept, MSD, Novartis and Phenex . He further received travel grants from Abbvie, Falk, Gilead and Intercept and unrestricted research grants from Albireo, Cymabay, Falk, Gilead, Intercept, MSD and Takeda. He is also co-inventor of a patent on the medical use of norUDCA. MLS: Advisory board: Alexion (former Wilson Therapeutics), Vivet Therapeutics, GMPO, Kadmon; Wilson's Disease Association (unpaid); Contracted research: Alexion, GMPO; Speaker: Gilead. HZ: has received honoratia for speaking and consulting Abbvie, BMS, Gilead, BMS, Novartis, Vifor, Pharmacosmos. JP,PM,CW and ASt have nothing to report.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

PF: study concept and design, critical revision of the manuscript for important intellectual content FP: writing of the manuscript, data collection; ASt, JP, PM, RS: data acquisition, KHW, HZ: data acquisition, critical revision of the manuscript for important intellectual content; MT,MS: critical revision of the manuscript for important intellectual content; CW: genetic tests

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2019.02.007.

References

[1] Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. NEJM 2018;378:1096–1106.

[2] Ma Y, Belyaeva OV, Brown PM, Fujita K, Valles K, Karki S, et al. HSD17B13 is a Hepatic Retinol Dehydrogenase Associated with Histological Features of Non-Alcoholic Fatty Liver Disease. Hepatology 2018; https://doi.org/10.1002/hep.30350.

[3] Pirola CJ, Garaycoechea M, Flichman D, Arrese M, San Martino J, Gazzi C, et al. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. J Lipid Res 2019;60(1):176–185.

[4] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, et al. Tissue-based map of the human proteome. Science 2015;3471260419.

[5] Rotroff DM, Pijut SS, Marvel SW, Jack JR, Havener TM, Pujol A, et al. Genetic Variants in HSD17B3, SMAD3, and IPO11 Impact Circulating Lipids in Response to Fenofibrate in Individuals With Type 2 Diabetes. Clin Pharmacol Ther 2018;103:712–721.

[6] Horiguchi Y, Araki M, Motojima K. 17beta-hydroxysteroid dehydrogenase type 13 is a liver-specific lipid droplet-associated protein. Biochem. Biophys. Res. Commun. 2008;370:235–238.

[7] Ferenci P, Stremmel W, Członkowska A, et al. Age, sex, but not ATP7B genotype effectively influences the clinical phenotype of Wilson disease. Hepatology 2018; https://doi.org/10.1002/hep.30280 (published online).

[8] Stättermayer AF, Traussnigg S, Dienes HP, Aigner E, Stauber R, Lackner K, et al. Hepatic steatosis in Wilson disease – role of copper and *PNPLA3* mutations. J. Hepatol 2015;63:156–163.

[9] Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. Final report of the proceedings of the working party at the 8th International Meeting on Wilson disease and Menkes disease, Leipzig/Germany, April 16-18,2001, 23. Liver International. p. 139–142.

[10] EASL Clinical Practice Guidelines. Wilson´s disease. J Hepatol. 2012;56:671–685.

[11] Roche-Sicot J, Benhamou JP. Acute intravascular hemolysis and acute liver failure associated as a first manifestation of Wilson's disease. Ann Intern Med 1977;86:301–303.

[12] Walshe JM. The acute haemolytic syndrome in Wilson's disease–a review of 22 patients. QJM 2013;106:1003–1008.

[13] Roche-Sicot J, Benhamou JP. Acute intravascular hemolysis and acute liver failure associated as a first manifestation of Wilson's disease. Ann Intern Med 1977;86:301–303.

[14] Dhawan A, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl 2005;11:441–448.

[15] Eisenbach Ch, Sieg O, Stremmel W, Encke J, Merle U. Diagnostic criteria for acute liver failure due to Wilson disease. World J Gastroenterol. 2007;13:1711–1714.

[16] Damsgaard J, Larsen FS, Ytting H. Reversal of acute liver failure due to Wilson's disease by a regimen of high-volume plasma exchange and penicillamine. Hepatology 2019; https://doi.org/10.1002/hep.30323.

[17] Hochstein P, Kumar S, Forman SJ. Lipid peroxidation and the cytotoxicity of copper. Ann. NY Acad. Scie 1980;355:240–248.

[18] Mufti AR, Burstein E, Csomos RA, Graf PC, Wilkinson JC, Dick RD, et al. XIAP Is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. Mol Cell 2006;21:775–785.

[19] Strand S, Hofmann WJ, Grambihler A, Hug H, Volkmann M, Otto G, et al. Hepatic failure and liver cell damage in acute Wilson's disease involve CD95 (APO-1/Fas) mediated apoptosis. Nat Med 1998;4:588–593.

[20] Kalita J, Kumar V, Misra UK. A Study on Apoptosis and Anti-apoptotic Status in Wilson Disease. Mol Neurobiol. 2016;53:6659–6667.
[21] Bruha R, Vitek L, Marecek Z, Pospisilova L, Nevsimalova S, Martasek P, et al. Decreased serum antioxidant capacity in patients with Wilson disease is associated with neurological symptoms. J Inherit Metab Dis 2012;35:541–548.
[22] Nagasaka H, Inoue I, Inui A, Komatsu H, Sogo T, Murayama , et al. Relationship between oxidative stress and antioxidant systems in the liver of patients with Wilson disease: hepatic manifestation in Wilson disease as a consequence of augmented oxidative stress. Pediatr Res 2006;60:472–477.
[23] Harrison PM, Wendo JA, Gimson AE, Alexander GJ, Williams R. Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. NEJM 1991;324:1852–1857.
[24] Chen G. The link between Hepatic Vitamin A Metabolism and Nonalcoholic Fatty Liver Disease. Curr Drug Targets 2015;16:1281–1292.
[25] Saeed A., Dullaart R.P.F., Schreuder T.C.M.A., Blokzijl H., Faber K.N.. Disturbed Vitamin A Metabolism in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients 2017;10(1) https://doi.org/10.3390/nu10010029.
[26] Kim SC, Kim CK, Axe D, Cook A, Lee M, Li T, et al. All-trans-retinoic acid ameliorates hepatic steatosis in mice by a novel transcriptional cascade. Hepatology 2014;59:1750–1760.