

Supplementary Results

Homozygous frame shift variant in *ATP7B* exon 1 leads to bypass of nonsense-mediated mRNA decay and to a protein capable of copper export

Amelie Stalke¹, Eva-Doreen Pfister², Ulrich Baumann², Marlies Eilers¹, Vera Schäffer¹, Thomas Illig^{1,3}, Bernd Auber¹, Brigitte Schlegelberger¹, Renate Brackmann⁴, Holger Prokisch^{5,6}, Simon Krooss⁷, Jens Bohne⁷, Britta Skawran¹

¹Department of Human Genetics, Hannover Medical School, Hannover, Germany

²Department of Kidney, Liver and Metabolic Disease, Division of Pediatric Gastroenterology and Hepatology, Hannover Medical School, Hannover, Germany

³Hannover Unified Biobank, Hannover Medical School, Hannover, Germany

⁴Department of Child and Adolescent Medicine, Klinikum Herford, Herford, Germany.

⁵Institute of Human Genetics, Helmholtz Center Munich, Neuherberg, Germany

⁶Institute of Human Genetics, Technical University Munich, Munich, Germany

⁷Department of Virology, Hannover Medical School, Hannover, Germany

Corresponding author:

Dr. Amelie Stalke, email: Stalke.Amelie@mh-hannover.de

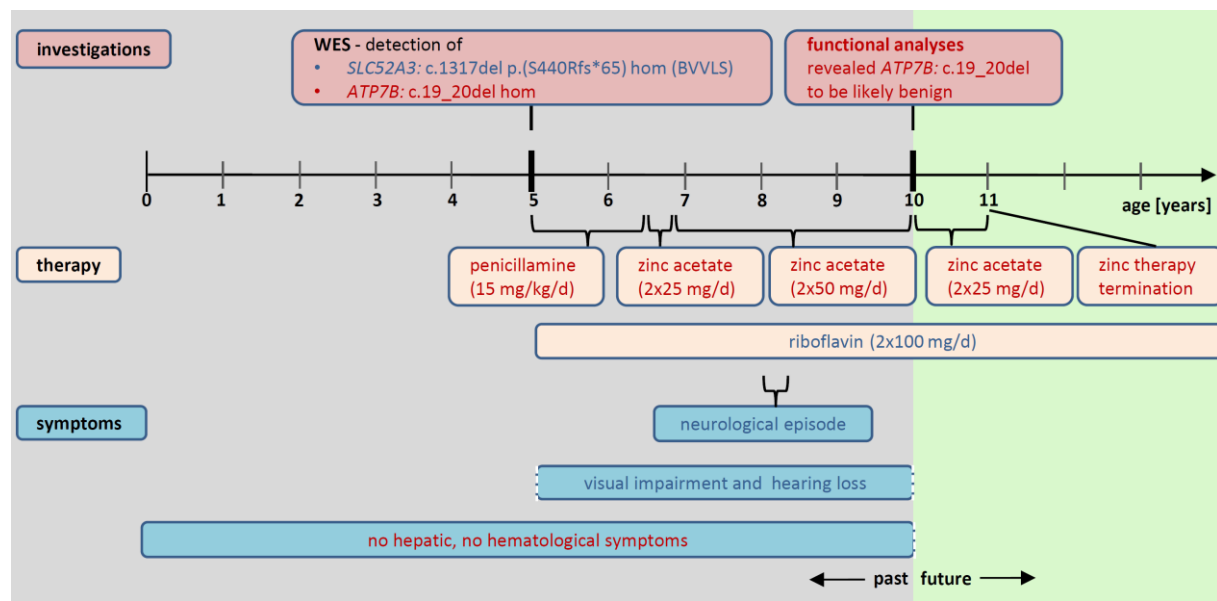


Fig. S1 Timeline of performed investigations, initiated therapies and symptoms of the index patient as well as planned therapy adjustment. Red font illustrates information in conjunction with the *ATP7B* variant c.19_20del, blue font illustrates information in conjunction with the *SLC52A3* variant c.1317del; BVVLS: Brown-Vialetto-Van-Laere syndrome, hom: homozygous.

Full size pictures of northern and western blots

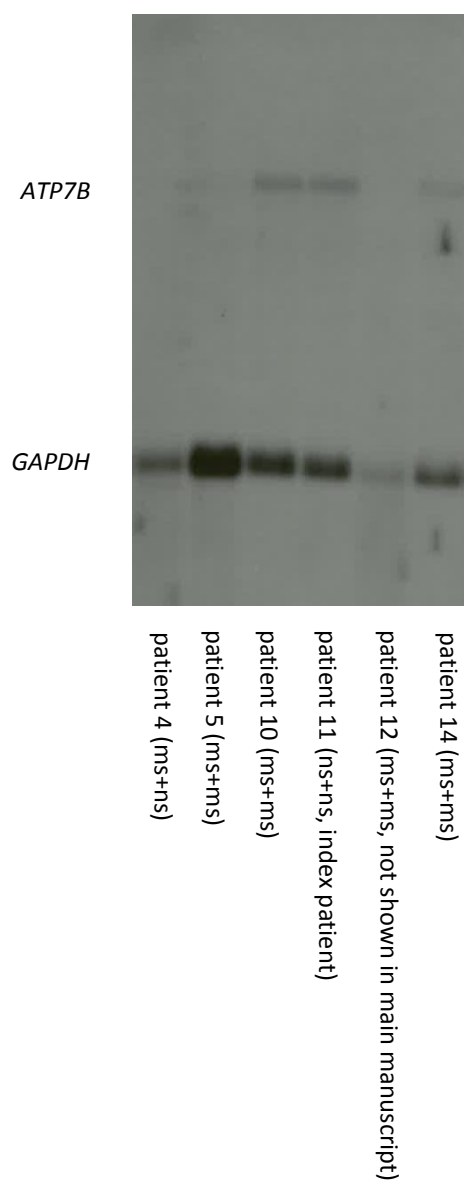


Fig. S2 Full size northern blot of RNA from liver tissue of exemplarily chosen WD patients (see Tab. S1); ms: missense variant, ns: nonsense or frameshift variant.

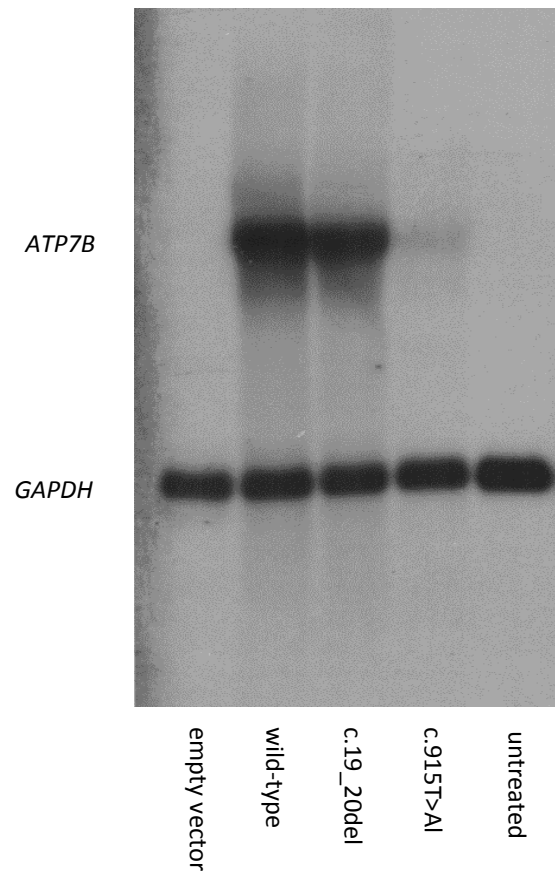


Fig. S3 Full size northern blot of RNA from HEK293T cells transfected with pcDNA3 empty vector, or *ATP7B* expression vectors pcDNA3_wild-type, pcDNA3_c.19_20del, pcDNA3_915T>A for 48 h or left untreated.

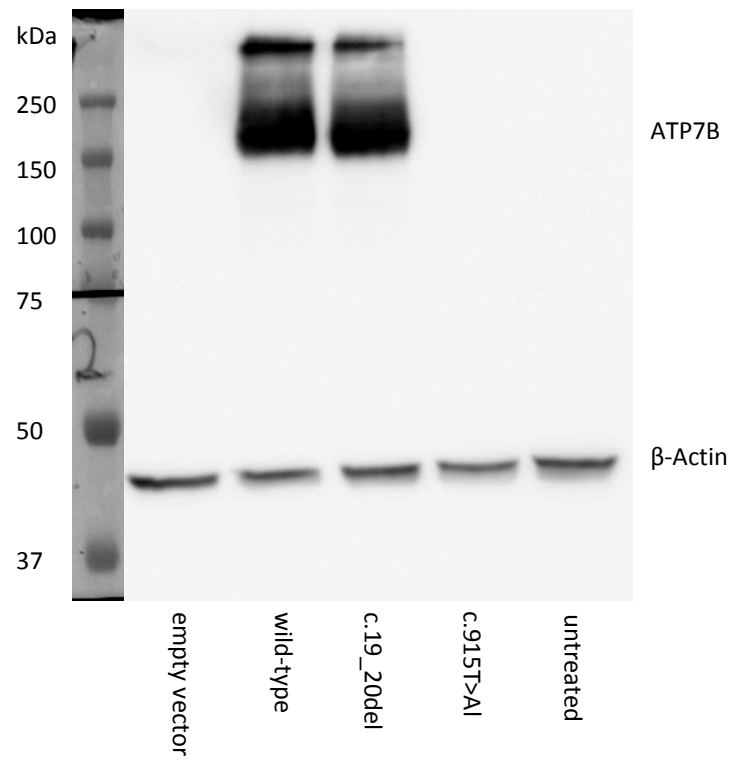


Fig. S4 Full size western blot of protein from HEK293T cells transfected with pcDNA3 empty vector or *ATP7B* expression vectors pcDNA3_wild-type, pcDNA3_c.19_20del, pcDNA3_915T>A for 48 h or left untreated.

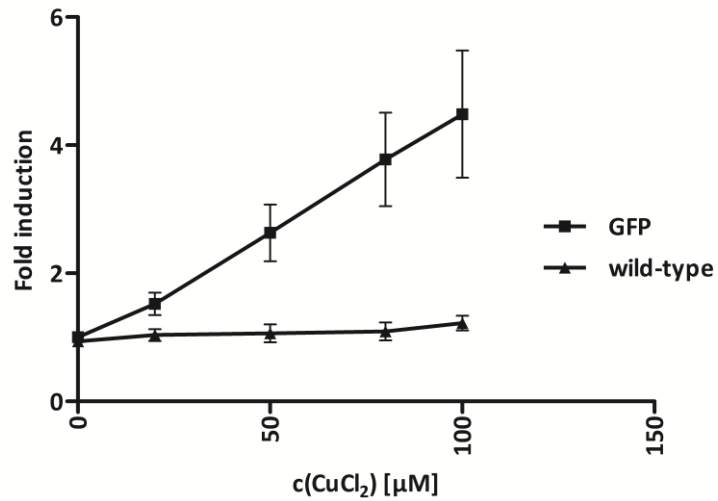


Fig. S5 Copper concentration-dependent luciferase induction under co-expression of pcDNA3_GFP or pcDNA3_wild-type. HEK293T cells were co-transfected with pGL3_4MRE, pRL-TK and pcDNA3_GFP or *ATP7B* expression vector pcDNA3_wild-type. After 24 h cells were treated with 0, 20, 50, 80 or 100 μM CuCl₂. Luciferase activity was measured after additional 24 h and normalized to renilla luciferase activity. Values were corrected for pcDNA3_GFP under basal copper condition (0 μM). Error bars show standard deviation.

Tab. S1 *ATP7B* variants of investigated Wilson disease patients and corresponding relative expression values in *ATP7B* qRT-PCR. Nomenclature according to HGVS (www.hgvs.org) for NCBI reference transcript NM_000053.3, consecutive exon numbering with c.1 as first nucleotide of exon 1.

patient	Detected <i>ATP7B</i> variants	Relative <i>ATP7B</i> expression in qRT-PCR
1	Ex8: c.2336G>A p.(W779*) heterozygous Ex14: c.3207C>A p.(H1069Q) heterozygous	1,68
2	Ex8: c.2222dupA p.(Y741*) heterozygous Ex18: c.3890T>A p.(V1297D) heterozygous	2,26
3	Ex14: c.3207C>A p.(H1069Q) homozygous	2,07
4	Ex2: c.915T>A p.(C305*) heterozygous Ex14: c.3207C>A p.(H1069Q) heterozygous	1,39
5	Ex8: c.2305A>G p.(M769V) homozygous	1,17
6	Ex18: c.3809A>G p.(N1270S) homozygous	2,78
7	Ex14: c.3207C>A p.(H1069Q) heterozygous Ex20: c.4054C>T p.(Q1352*) heterozygous	1,54
8	Ex16: c.3424C>T p.(Q1142*) homozygous	0,51
9	Ex10: c.2532delA p.(V845Sfs*28) homozygous	0,56
10	Ex18: c.3809A>G p.(N1270S) homozygous	6,99
11	Ex1: c.19_20delCA p.(Q7Dfs*14) homozygous	6,71
12	Ex14: c.3207C>A p.(H1069Q) heterozygous Ex7: c.2108G>A p.(C703Y) heterozygous	1,79
13	Ex18: c.3809A>G p.(N1270S) homozygous	6,69
14	Ex18: c.3809A>G p.(N1270S) homozygous	4,24
15	Ex14: c.3207C>A p.(H1069Q) homozygous	1,91

Tab. S2 Potential new start codons with amino acid localization and corresponding protein domain

		Downstream ATG in exon 2									
ATG	original	1	2	3	4	5	6	7	8	9	10
kDa	157.29	153.66	150.17	147.76	144.53	141.08	135.22	128.55	117.95	116.73	112.49
AA position	1	33	67	89	119	152	204	266	368	380	420
Domain ₁	/	/	MBD1	MBD1	MBD1	MBD2	MBD2	MBD3	MBD4	MBD4	Linker 4

MBD: metal-binding domain, AA: amino acid

Supplementary Results - References

1. Arioiz C, Li Y, Wittung-Stafshede P: The six metal binding domains in human copper transporter, ATP7B: molecular biophysics and disease-causing mutations. *Biometals* 2017; **30**: 823-840.