METHODS

Genetic testing

WES was performed using the SureSelect Clinical Research Exome v2.0 enrichment kit (Agilent Technologies, Santa Clara, CA). Variant annotation and filtering were performed as previously described (1). Variants were filtered according to: effect on protein and transcript; patient phenotype (www.human-phenotype-ontology.org); inheritance model; minor allele frequency in general population. Candidate variants – classified according to ACMG guidelines (2) – should have a consistent pathogenic mechanism. Pathogenic variants were confirmed in the proband and parents by Sanger sequencing using a second independent DNA sample.

Pathology studies

For optical microscopy, liver tissue underwent standard hematoxylin/eosin and Masson trichrome stains.

For transmission electron microscopy, after fixation, inclusion, and toluidine/sodium tetraborate staining of semithin sections, blocks were thin-sectioned at 85 nm, and stained in saturated uranyl acetate in 99% alcohol and Reynold’s lead citrate.

RNA studies

Upon consent, liver tissue was collected from hepatectomy in the patient and in three age-matched children undergoing liver transplant (LT) for biliary atresia (BA), and from an age-matched donor following whole LT, and subjected to gene expression analysis using QuantSeq 3’-mRNA sequencing. RNA extraction, amplification and cDNA sequencing were performed as previously described (3). The sequence reads were trimmed using the Trim Galore software (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), and aligned on the hg19 reference sequence using STAR. Genes expression was
determined with htseq-count using the Gencode v19 gene model. Differential expression analysis was performed using EdgeR. A false discovery rate (FDR) $< 0.05$ was used as a threshold.

WD-related human homologue genes were extrapolated from a genome-wide mRNA profiling in $ATP7B$-deficient mice (4).

For gene ontology enrichment analysis, genes significantly up- or down-regulated at least 2-fold were clustered according to the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg) and compared with three system biology analysis in rodent models of WD (5).
References


