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

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