Supplement

Methods

Whole-exome sequencing (WES)

Libraries for WES were prepared with SureSelectXT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library v6 (Agilent, Santa Clara, CA, USA) according to the manufacturer's protocols and sequenced in paired-end 100-bp mode using an Illumina HiSeq4000 sequencing instrument (Illumina, San Diego, CA, USA). FreeBayes (arXiv:1207.3907) was used for variant detection. All detected variants were manually curated using the Integrated Genome Viewer. Common SNPs at a certain population frequency were excluded (>1% in 1000genomes and >1% in ExAC).

Immunoblotting

RBC membrane extracts (50 µg protein) were loaded on SDS-polyacrylamide gels, transferred onto polyvinylidene difluoride membranes (BioRad, Milan, Italy) and incubated with the following antibodies: rabbit anti-ABCB6 (1:500; SAB1300078, Sigma Aldrich, Milan, Italy); mouse anti-β-actin antibody (1:12000; Sigma Aldrich, Milan, Italy) was used as a control for equal loading. Incubation with HRP-conjugated anti-rabbit Ig (1:4000) (GE Healthcare, UK) and HRP-conjugated anti-mouse Ig (1:4000) (GE Healthcare, UK) was performed and labeled bands were visualized (Supersignal West Pico Chemiluminescent Substrate Kit, ThermoScientific, Miami, USA). Densitometric analysis was performed with the BioRad Chemidoc using Quantity One software (BioRad).