Supplementary

MATERIALS AND METHODS

Observational clinical studies (case report or case series report) do not require approval by the ethics committee according to AIFA (Italian Medicines Agency) statement 20-03-2008 published in G.U. n. 76 of 31-3-2008. All patients signed the informed consent for genetic testing which include the authorization of the processing of clinical and genetic data for research purposes in compliance with local (circ.reg 28.05.2013) and national legislations (General Authorization n°8 of Garante Privacy) and according to General Data Protection Regulation (Regulation (EU) 2016/679 of the EU Parliament and of the Council of 27 April 2016) and to the Helsinki Declaration.

Genetic Analysis

Next generation sequencing (NGS)

Genomic DNA from whole EDTA blood was extracted using the automated Maxwell® RSC Instrument (Promega Corporation, Madison, USA) and DNA samples were quantified by Infinite® 200 PRO Nano-Quant (Tecan, Männedorf, Switzerland). In order to identify potential pathogenic variants a custom multi-gene panel (panel available on request) for ADTKD and ADPKD was designed using Ion Ampliseq Designer (Life Technologies, CA, USA). NGS analysis was performed using Ion Torrent Personal Genome Machine (PGM) System (LifeTechnologies Ltd., Paisley, UK) and raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37), was performed using the Torrent Suite Software version 5.5 (Thermo Fisher Scientific, Waltham, MA, USA). Sanger sequencing was performed in order to validate all the variants classified as pathogenic, likely pathogenic and VUS.

Multiplex ligation-dependent amplification (MLPA) analysis.

Deletion and duplication analysis of HNF1B was performed using a commercial MLPA kit (SALSA MLPA® Probemix P241, MRC Holland, Amsterdam, The Netherlands) and the results were analyzed using CoffalyserTM Software following the manifacture's protocol.

Deletion and duplication analysis of PKD1 and PKD2 was performed using two commercial MLPA kits (MLPA P351-C1 and P352-D1 probemixes; MRC-Holland, The Netherlands). The probemixes used for this test do not contain probes for exons 1, 2, 4, 8, 17, 24, 28, 32, 34 and 45, therefore a deletion or duplication of a single exon in these regions cannot be detected.

Array-based Comparative Genomic Hibridization (CGH-array).

In family 6 (Fig.1) CGH-array analysis was performed using a commercially available oligonucleotide microarray containing about 180000 probes (PerkinElmer oligo genome-wide CGX-HD

Array 180K) with an average effective resolution of about 60 kb in the critical regions and 120 kb in the backbone. Data were analyzed and displayed with Genoglyphix analysis software (PerkinElmer, Inc., Spokane, WA, USA).

Whole Exome sequencing (WES).

WES was performed in individuals I-1 of family 6. We obtained an average exome coverage of 40X. After quality control, we excluded variants already listed in polymorphism repositories (db-SNP) with MAF greater than 0.01 or in Exome Variant Server with MAF greater than 0.001, or identified in 15 exomes from non-related, healthy Italian subjects.

Variant interpretation

In identified nonsense variants the premature termination codons (PTCs) containing transcripts could be degraded by the mRNA surveillance pathway termed nonsense-mediated mRNA decay (NMD) and the remaining wild-type version may be haploinsufficient.

Interpretation of missense variants was based on the following ACMG criteria: presence in the population database (gnomAD Browser), mutation previously reported in HNF1B deficiency, based on literature and gene-specific database (Human Genome Mutation Database [HGMD]), de novo occurrence and familial cosegregation analysis, predictive algorithms of pathogenicity for missense variants.