eMethods

Participants and data collection

We enrolled 94 unrelated PKD patients who were negative for both *PRRT2* and *TMEM151A* variants[1–3] from the Second Affiliated Hospital of Zhejiang University School of Medicine between January 2015 and July 2021. The inclusion criteria were as follows: an identified kinesigenic trigger for the attacks; no loss of consciousness or pain during attacks. The study was approved by the ethics committee of the Second Affiliated Hospital. All patients or their guardians provided written informed consents. For patients detected to carry 16p11.2 microdeletion, their medical records were reviewed and clinical data were extracted, including age at onset, gender, height, weight, family history, nature of attacks, comorbidity, drug response and the result of electroencephalogram (EEG) and brain magnetic resonance imaging (MRI). Information related to motor and language developmental history, learning ability, social behavior and medication usage in the past month was obtained from their guardians by telephone follow up. Clinical information on previously reported patients was recorded according to the original article.

Whole-exome sequencing

Blood samples were obtained from recruited patients and available parents. Genomic DNA was isolated using the QIAamp® DNA Blood Mini Kit (QIAGEN, Germany), and then fragmented, subjected to library construction and captured by Agilent SureSelect Human All Exon (V7) (Agilent Technologies, USA) or Roche NimbleGen V3 (64M) (Roche NimbleGen, Inc., USA). The sequencing was performed on the Illumina NovaSeq 6000 platform or the Illumina X-ten platform (Illumina, USA). Raw data was obtained using Illumina pipeline software. The junction sequences, low-quality reads were filtered out to derive clean data, which was aligned to the human

genome reference (hs37d5) with the Burrows Wheeler Aligner software. Single nucleotide variants (SNVs) and small insertions/deletions (indels) were called using the Genome Analysis Toolkit software and annotated with ANNOVARA. CNV detection was performed using ExomeDepth or EXCAVATOR software.[4,5] Whole-exome sequencing (WES) data of test and control samples were provided to the software in a BAM file format. Controls were matched by gender and the enrichment kit used. The whole-exome sequencing was performed at the XiangYin Biotechnology Co. Ltd (Hangzhou, China) and the Genergy Biotechnology Inc. (Shanghai, China).

Quantitative PCR

To verify the copy number variants suggested by the WES data, we first detected the copy number of PRRT2 using quantitative PCR (qPCR) with TaqMan Copy Number Assays. The pre-designed probes targeted *PRRT2* (Hs00931442_cn, chr.16:29813743 on GRCh38) and a reference gene *RNase P* (Cat.4403326, chr.14:20343370 on GRCh38) were purchased from Applied Biosystems (Life Technologies, Inc., USA). Genomic DNA was diluted to a concentration of 5 ng/μL and qPCR was performed on the StepOnePlus™ System (Applied Biosystems, USA) according to the manufactory's instructions. There were three replicates for each DNA sample, a non-template control and two controls in each experiment. Data were exported and further analyzed by the CopyCaller software version 2.1 (Applied Biosystems, USA) with the default parameters. Experiment was repeated for samples showing copy number changes of PRRT2.

Low-coverage whole-genome sequencing

Once the *PRRT2* deletion was confirmed by qPCR, low-coverage whole-genome sequencing (CNV-seq) with a read depth of 2–3X was performed to further verify the 16p11.2 microdeletion. The procedure of library construction, reads sequencing and

alignment was the same as what described above. Copy number changes were analyzed by R package - DNAcopy. Low complexity regions were filtered out. Then, mapped reads were categorized into sliding windows, which were 10kb in length. The number of reads in each sliding window was calculated and then transformed into the coverage of the window after the population-scale normalization and GC-content correction. After that, the binary segmentation algorithm was used to identify the potential CNV regions. The low-coverage whole genome sequencing was performed at the XiangYin Biotechnology Co. Ltd (Hangzhou, China).

Microsatellite analysis

Microsatellites were analyzed using the Investigator 24plex QS Kit (QIAGEN, Germany) according to the manufactory's instructions. Samples were analyzed on an ABI 3500XL Dx Genetic Analyzer and processed using GeneMapper *ID-X* software (Applied Biosystems). Paternity Index (PI) was calculated for each autosomal genetic locus. Combined paternity index (CPI) was obtained by multiplying individual PIs together. The allele frequencies for calculation were obtained from the "Excel file of 1036 revised allele frequencies" on STRBase website (https://strbase.nist.gov/NISTpop.htm). Probability of paternity was calculated as CPI/ (1 + CPI).

References

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4. Plagnol V, Curtis J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*. 2012; 28(21):2747–2754.
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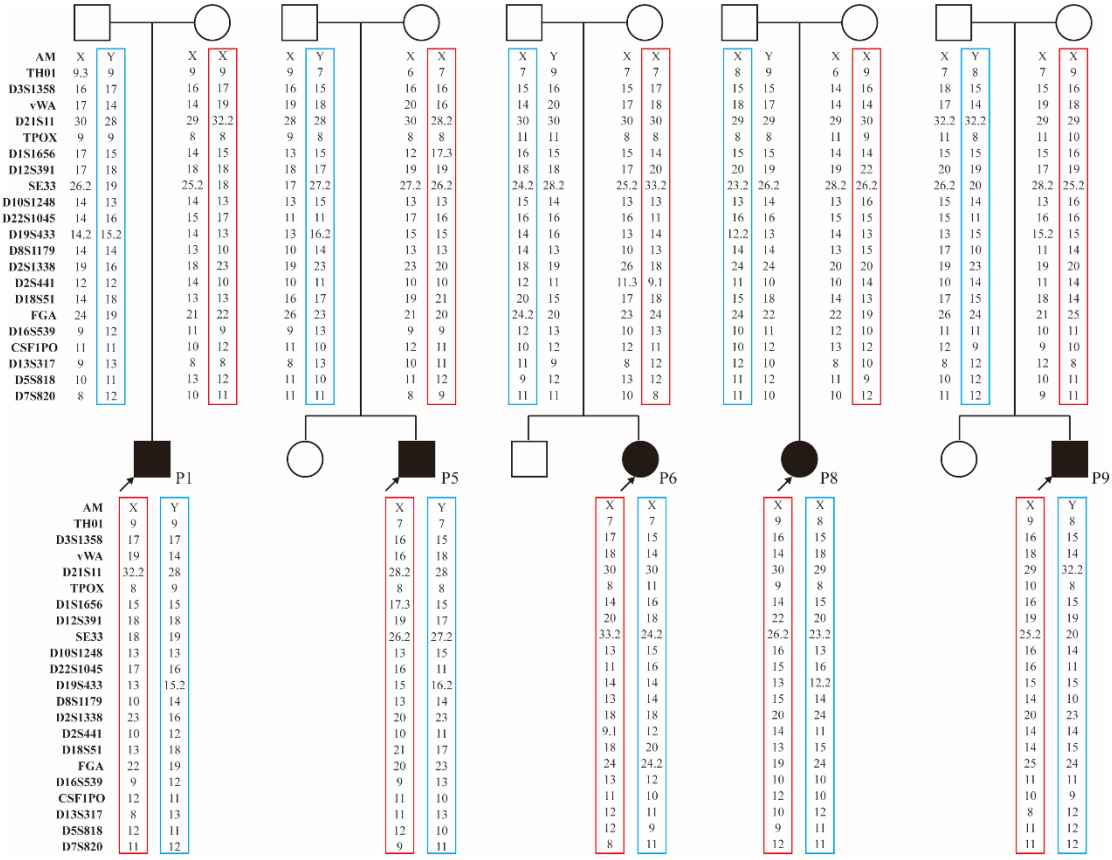
eTable 1 The range of 16p11.2 microdeletions analyzed with whole-exome sequencing data

Patient	CNV range (hg 19)	Size	One-copy genes indicated by CNV analysis
P1	Chr16: 29580569-30198321	617.8kb	SPN/ZG16/KIF22/PRRT2/MVP/SEZ6L2/ASPHD1/KCTD13 /TMEM219/TAOK2/HIRIP3/INO80E/DOC2A/C16orf92/FAM57B /ALDOA/PPP4C/TBX6/YPEL3/GDPD3/MAPK3/CORO1A
P2	Chr16: 29571443-30199160	627.7kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/MVP/SEZ6L2 /ASPHD1/KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/DOC2A /C16orf92/FAM57B/ALDOA/PPP4C/TBX6/GDPD3/CORO1A
P3	Chr16: 29571443-30199160	627.7kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/PAGR1/MVP/SEZ6L2/AS PHD1/KCTD13/TAOK2/HIRIP3/INO80E/C16orf92/FAM57B/ALDO A/PPP4C/TBX6/YPEL3/MAPK3/CORO1A
P4	Chr16: 29557415-30129545	572.1kb	SPN/QPRT/ZG16/KIF22/PRRT2/MVP/CDIPT/SEZ6L2/ASPHD1 /KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/DOC2A/C16orf92 /FAM57B/ALDOA/PPP4C/TBX6/YPEL3/GDPD3/MAPK3
P5	Chr16: 29557415-30199610	642.2kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/PAGR1/MVP/SEZ6L2/AS PHD1/KCTD13/TAOK2/HIRIP3/INO80E/DOC2A/C16orf92 /FAM57B/ALDOA/PPP4C/TBX6/YPEL3/MAPK3/CORO1A
P6	Chr16: 29557415-30199610	642.2kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/PAGR1/MVP/CDIPT /SEZ6L2/ASPHD1/KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/DO C2A/C16orf92/FAM57B/ALDOA/PPP4C/TBX6/YPEL3/GDPD3/MA PK3/CORO1A
P7	Chr16: 29571443-30199610	628.2kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/PAGR1/MVP/CDIPT /SEZ6L2/ASPHD1/KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/DO C2A/C16orf92/FAM57B/ALDOA/PPP4C/TBX6/YPEL3/GDPD3/MA PK3/CORO1A
P8	Chr16: 29449240-30199846	750.6kb	BOLA2/BOLA2B/SLX1A/SLX1B/SULT1A3/SULT1A4/NPIPB12 /SPN/QPRT/C16orf54/ZG16/KIF22/MAZ/PRRT2/PAGR1/MVP/CDIP T/SEZ6L2/ASPHD1/KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/D OC2A/C16orf92/FAM57B/ALDOA/PPP4C/TBX6/YPEL3/GDPD3/M APK3/CORO1A
P9	Chr16: 29675050-30199897	524.8kb	SPN/QPRT/C16orf54/ZG16/KIF22/PRRT2/PAGR1/MVP/CDIPT /SEZ6L2/ASPHD1/KCTD13/TMEM219/TAOK2/HIRIP3/INO80E/DO C2A/C16orf92/FAM57B/ALDOA/PPP4C/TBX6/YPEL3/GDPD3/MA



eFigure 1. Paternity testing result of our five families using microsatellite analysis. Based on the analyses of autosomal STR loci listed above, the paternity of probability of each family was more than 99.99%. P1 represents the patient 1 in the text, and so on. Circle = female; square = male; open symbol = unaffected; filled symbol = affected; arrow = proband.