

Supplementary methods

Patients

From November 2013 to September 2015, 55 polycystic kidney disease (PKD) patients without past history of stroke at the National Taiwan University Hospital were prospectively enrolled for brain magnetic resonance imaging (MRI) with susceptibility-weighted imaging (SWI). All patients received either abdominal computed tomography or MRI to confirm the diagnosis of PKD. Criteria for the diagnosis included at least two unilateral or bilateral cysts in persons younger than 30 years of age, at least two cysts in each kidney in persons 30 to 59 years of age and at least four cysts in each kidney in persons 60 years of age or older.¹ Demographic data, comorbidities, renal condition, blood platelet count, serum level of alanine aminotransferase, the usage of anti-platelet agent, and family history of each patient were comprehensively reviewed and recorded. The diagnosis of hypertension was defined as either treatment with antihypertensive drugs; or three home measurement of either a mean systolic blood pressure ≥ 130 mm Hg or a mean diastolic blood pressure ≥ 80 mm Hg.⁴ The diagnosis of dyslipidemia was defined as either treatment with lipid lowering agents; or either serum total cholesterol level >200 mg/dL or low density lipoprotein >130 mg/dL. We calculated the estimated glomerular filtration rate (eGFR) using the simplified Modification of Diet in Renal Disease equation as follows: $eGFR (mL/min/1.73 m^2) = 186 \times \text{serum creatinine (Scr)}^{-1.154} \times \text{Age}^{-0.203} \times 0.742$ (if female).⁵ This study was approved by the hospital Research Ethics Committee (201405049RIND and 201502049RINB). All patients provided written informed consent to participate.

Forty-five patients who visited the neurology outpatient clinics of National Taiwan University Hospital from July 2015 to March 2019 were chosen as control subjects. These patients had no prior stroke or family history of PKD. All received a brain MRI study and renal function test. Demographic data and comorbidities were recorded. The final diagnosis of these control subjects were primary headache (55.6%), non-specific dizziness (20.0%), anxiety (15.6%), transient global amnesia (4.4%), trigeminal neuralgia (2.2%), and idiopathic hemifacial spasm (2.2%). Only one patient had definite chronic kidney disease (eGFR=32 mL/min/1.73 m²) and his abdominal echo showed no evidence of polycystic kidneys.

Brain magnetic resonance imaging

All patients received a non-contrast brain MRI on a 3-Tesla MR scanner (Verio, TIM or mMR, Siemens, Erlangen, Germany). The imaging protocol consisted of axial T1-weighted, T2-weighted,

T2-fluid attenuated inversion recovery (FLAIR) images, SWI (covering the whole brain) and time-of-flight magnetic resonance angiography (for the intracranial vessels) sequences. Twenty-two PKD patients (40%) received another follow-up brain MRI study over a period ranging from 14 to 60 months.

We obtained the SWI using a T2*-weighted gradient echo sequence with flip angle 15°, TR/TE=28/20 msec, matrix number=221×320, FOV=23 cm, and slice thickness=1.6 mm. SWI and minimum intensity projection images, acquired by in-line post-processing of the magnitude and phase images, were used to evaluate the imaging findings in this study. Cerebral microbleeds (CMBs) were defined as small, round or ovoid hypointensities with a diameter less than 1 cm on SWI.³ We further categorized the location of CMBs into the lobar (cortical or cortical-subcortical areas in the fronto-parieto-temporo-occipital lobes) and deep regions (basal ganglion, thalamus and infratentorial areas).⁶ The intraobserver and interobserver reliability (κ) was 0.84 and 0.70 in the determination of lobar CMB count and 0.76 and 0.72 in the determination of deep CMB count.⁷

Other neuroimaging markers of small vessel disease were rated according to the Standards for Reporting Vascular changes on neuroimaging (STRIVE) criteria [3], and evaluated as described previously.⁶ Briefly, white matter hyperintense (WMH) lesions were evaluated on FLAIR images using Fazekas scale. The WMH from moderate to severe was defined as Fazekas scale ≥ 2 .^{6,8} Lacunes were defined as "round or ovoid, subcortical, fluid-filled (similar signal as cerebrospinal fluid) cavity, ranging 3 to 15 mm in diameter",^{6,9} and were evaluated in the supratentorial region, pons and cerebellum, respectively. Enlarged perivascular spaces (EPVSs) were defined as sharply delineated structures on T2-weighted imaging, measuring <3 mm in diameter followed by the course of perforating or medullary vessels. The number of EPVS (on the side of the brain with more severe involvement) was counted in basal ganglion and centrum semiovale. We pre-specified a dichotomized classification of EPVS degree as high (score >20) or low (score ≤ 20).^{6,10}

Genotyping with next-generation sequencing

A total of 49 patients with PKD underwent the genetic test using next-generation sequencing (NGS) to screen the entire *PKD1* and *PKD2* genes for mutations while six patients refused to take the test. Briefly, blood samples were collected and genomic DNA (gDNA) was extracted using a Gentra Puregene Blood Kit (QIAGEN, Frederick, MD, USA) according to the manufacturer's protocol. The gDNA was fragmented to about 800 base pairs using Covaris (Covaris Inc., Woburn, MA, USA). The base pairs were then measured by Agilent Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA). Target enrich-

ment was achieved using a customized NimbleGen SeqCap EZ Choice Kit (Roche NimbleGen Inc., Madison, WI, USA). NGS experiments were performed using Illumina MiSeq at the Laboratory of Molecular Genetic Diagnostics, National Taiwan University Hospital (<http://www.genetics-core-ntuh.tw/>). The bioinformatics analysis pipeline was essentially the same as described in our previous work.¹¹ All pathogenic variants detected through the NGS pipeline were further confirmed using the

classical Sanger sequencing method. A genotype-phenotype correlation study reported that both *PKD1* and *PKD2* patients were at risk of intracranial aneurysm and the 5' mutations were more commonly associated with intracranial aneurysm than 3' mutations in the *PKD1* gene.¹² Therefore, we also compared the percentage of patients having CMB between those with mutation at different *PKD1* gene regions (5':1–6,000 vs. 3':6,000–12,000).