Novel compound heterozygous mutation of the *diacylglycerol kinase epsilon* gene and membranoproliferative glomerulonephritis: a case report

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Case report

A 10-year-old Chinese boy first presented in June 2015 at the age of 4 years with steroid-resistant nephrotic syndrome. He had been taking full-dose prednisolone at 60 mg/m^2/day for 4 weeks. Renal biopsy confirmed immune complex–mediated membranoproliferative glomerulonephritis (MPGN) with predominant immunoglobulin M (IgM) and scanty IgG or C3 deposits. Cyclosporin A, a calcineurin inhibitor (CNI), was introduced and successfully brought the disease into partial remission. His urine protein–to-creatinine ratio (UPCR) reduced from 13.9 mg/mg to 0.95 mg/mg after 2 months. He remained in partial remission for 4 years with cyclosporin A and low-dose prednisolone. He showed a urinary relapse following discontinuation of cyclosporin A in 2020 with a rebound in UPCR from 0.64 mg/mg to 1.57 mg/mg (Table).

Renal biopsy was repeated and revealed mild-to-moderate global mesangial cell proliferation and a few clusters of arterioles showing hyaline arteriolosclerosis with a peripheral and segmental distribution (Fig). Of note, there was evidence of mesangiolysis with loss of argyrophilic basement membrane material in a segmental pattern. The direct immunofluorescence portion showed finely granular deposits of IgA (2+), IgG (3+), IgM (2+), C1q (3+), and C3 (+) in a diffuse global and capillary distribution. Electron microscopy showed focal fusion of podocyte foot processes, splitting of glomerular basement membrane in association with mesangial cell interposition and scattered subendothelial and intramembranous dense electron deposits in keeping with immune complex. The features were indicative of immune complex–mediated MPGN with evidence of CNI toxicity. The presence of segmental mesangiolysis was suggestive of previous endothelium injury, possibly an episode of glomerular thrombotic microangiopathy (TMA).

Next-generation sequencing was performed and detected two mutations in the *diacylglycerol kinase epsilon* (*DGKE*) gene, including a c.1068_1071del p.(Asn356Lysfs*6) frameshift mutation and a c.1282_1284+18del deletion. Sanger sequencing of the mother detected heterozygous *DGKE* gene c.1068_1071del p.(Asn356Lysfs*6). The father was unavailable for genetic analysis. In view of these results, the two *DGKE* variants were likely to act in trans in the patient. According to the American College of Medical Genetics/Association for Molecular Pathology classification, these two variants are considered pathogenic. Additional whole-exome sequencing revealed no significant variants in genes related to monogenic lupus.

Throughout the course of the disease, his blood tests showed no features of haemolytic uraemic syndrome (HUS). Apart from a mildly elevated lactate dehydrogenase, his complete blood count, blood smear and haptoglobin were normal. Complement function testing showed no evidence of complement activation. He was commenced on an angiotensin-converting enzyme inhibitor, prednisolone, and mycophenolate mofetil. He responded promptly with marked improvement in proteinuria: UPCR decreased to 0.36 mg/mg within 1 month. At last follow-up, his UPCR was 0.24 mg/mg with normal kidney function (estimated glomerular filtration rate = 116 mL/min/1.73 m^2).

Discussion

We report the first Chinese patient with *DGKE* nephropathy who presented as nephrotic syndrome and immune complex–mediated MPGN. Importantly, the patient responded well to immunosuppressive agents including CNI and mycophenolate mofetil. To the best of our knowledge, the pathogenic variants in this case are the first reported in *DGKE*-related nephrotic syndrome.

TABLE. Blood and urine test results of the patient

	First presentation (2015)	At urinary relapse (2020)	Normal range
Biochemical abnormalities for nephrotic syndrome			
Urine protein–to-creatinine ratio, mg/mg	13.9	1.57	<0.2
Urea, mmol/L	6.4	5.5	2.7-7.2
Creatinine, μmol/L	41	43	39-57
Albumin, g/dL	20	40	35-50
Total cholesterol, mmol/L	9.6	/	2.9-5.4
Triglyceride, mmol/L	3.24	/	<1.7
LDL, mmol/L	7.07	/	<2.6
Autoimmune and infection screen			
C3, g/L	1.17	1.38	0.7-2.06
C4, g/L	0.19	0.22	0.11-0.61
RF	Negative	/	
Anti-GBM	Negative	/	
Anti-dsDNA	Negative	80	≤50
Anti-ENA	/	Negative	
ANCA	/	Negative	
HBsAg	Negative	Negative	
Anti-HCV	Negative	Negative	
Workup for haemolytic uraemic syndrome			
Haemoglobin, g/dL	15.3	13.3	10.7-13.4
White cell count, /L	12.8 × 109	6.8 × 109	4.31-11 × 109
Platelets, /L	266 × 109	299 × 109	206-369 × 109
Blood film	/	No schistocytes	
Haptoglobin, g/L	/	0.95	0.08-1.55
LDH, IU/L	/	288	≤270
Complement activation	/	No	

Abbreviations: ANCA = antineutrophil cytoplasmic antibodies; dsDNA = double stranded DNA; ENA = extractable nuclear antigen; GBM = glomerular basement membrane; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; LDH = lactate dehydrogenase; LDL = low-density lipoprotein; RF = rheumatoid factor

Diacylglycerol kinase epsilon gene is important in the regulation of thrombotic status and is present in podocytes, platelets, and endothelium. A loss-of-function in the *DGKE* gene is associated with a prothrombotic state, leading to episodes of HUS that are complement-independent.[1] Interestingly, a subset of patients develop MPGN with nephrotic syndrome.[2] Along with the new classification, it is believed that chronic microangiopathy often results in a form of MPGN that is neither immune complex–mediated nor complement-mediated.[3] The exact mechanism of *DGKE* mutations leading to MPGN remains unknown.

Azukaitis et al[4] reported 44 cases of *DGKE* nephropathies, including 33 cases with HUS, nine cases with MPGN/nephrotic syndrome, and two rare cases with a mixed HUS/MPGN presentation. Among the nine reported cases of *DGKE*-MPGN, three other pathogenic variants were identified. Mutations of p.Gln43*, p.IVS5-2a and p.Thr204Glnfs*6 were found in four, three and two of the patients, respectively. Interestingly, the first case of *DGKE* nephropathy confirmed in our territory-wide next-generation sequencing cohort had nephrotic syndrome and MPGN.

The pathology of renal biopsy in this child is particularly interesting in terms of the presence of TMA features and an immune complex–mediated MPGN picture. The presence of mesangiolysis signifies endothelial injury such as TMA. These lesions might be caused by the underlying disease process related to *DGKE* mutations. Of note, about half of the patients with *DGKE*-HUS recovered spontaneously from the initial TMA episode

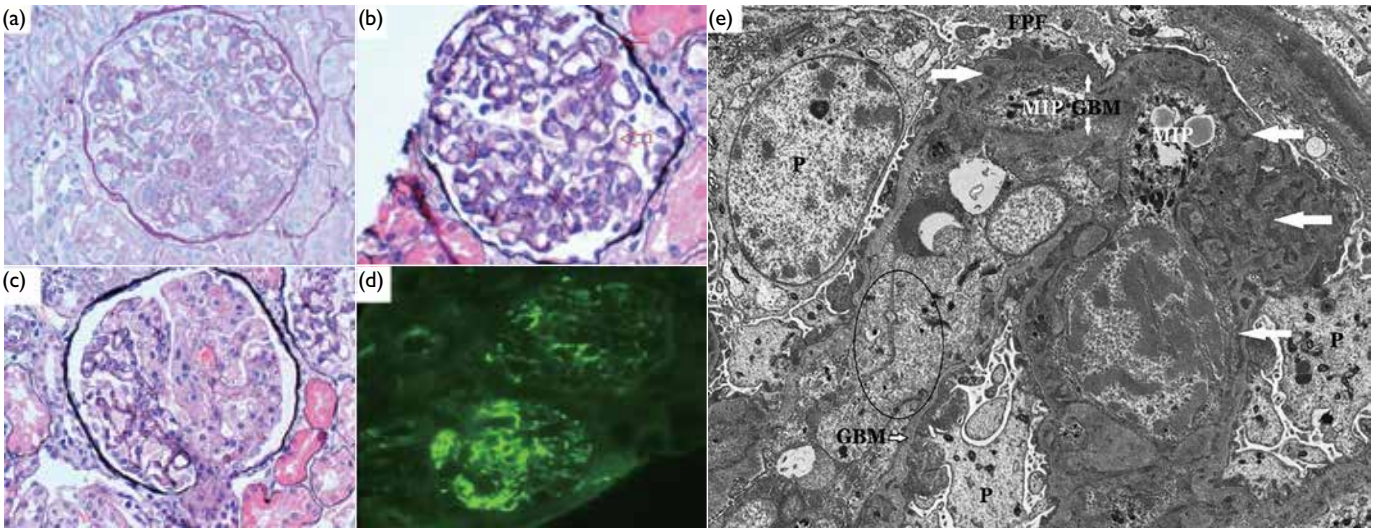

FIG. (a) Representative glomerulus showing mesangial proliferative glomerulonephritis pattern of changes with lobular accentuation of glomerular tuft and mild global mesangial cellular proliferation and thickened capillary wall (periodic acid–Schiff stain, original magnification ×400). (b) Representative glomerulus showing mesangial proliferative glomerulonephritis pattern of changes with lobular accentuation of glomerular tuft and mild global mesangial cellular proliferation and tram-track split capillary wall (arrow) [methenamine silver stain, original magnification ×400]. (c) Occasional glomerulus showing evidence of mesangiolysis evident by loss of argyrophilic mesangial material on the right part of the glomerulus. Such mesangiolysis may indicate previous injury of thrombotic microangiopathy (TMA) as there is no active TMA (methenamine silver stain, original magnification ×400). (d) Direct immunofluorescence study showing global mesangial and capillary deposit of immunoglobulin G in a fine granular pattern (original magnification ×400). (e) Electron microscopy. An inverted u-shaped glomerular capillary is present in the centre of the field surrounded by a nucleated podocyte (P) on the left upper corner and other podocytes in the middle and right side. Focal foot process fusion, denoted as FPF in the upper centre, is noted. The lumen of the capillary tuft is denoted by the oval circle on the left side of the field and this part is occupied by the cytoplasm of the endothelial cells. The glomerular basement membrane (GBM, small white arrow on the left lower side) is normal at this region. In the upper part of the capillary loop, there are two areas of mesangial interposition (MIP) with split and thickened GBM (two small white arrows). Large white arrows indicate the small intramembranous and subendothelial immune-complex dense deposits. The electron microscopy features are typical of immune complex–mediated membranoproliferative glomerulonephritis

without any HUS-specific treatment.[4] A potential explanation of this observation in our patient is that an episode of subclinical TMA had resolved on its own. Another interesting finding was the positive immunofluorescence on renal biopsy, not commonly seen in TMA-related MPGN. The immune-complex deposition in our patient could not be explained by either autoimmune disease or hepatitis. Although he had an isolated elevation of anti–double stranded DNA that occurred only on disease relapse, a confirmed diagnosis of systemic lupus could not be excluded. Whole-exome sequencing revealed no clinically significant variants in the monogenic lupus genes. Whether or not the finding of immune complex deposition at a histological level was an event secondary to the genetic mutation remains unknown. Clinicians need to be mindful of such a discrepant genetic finding and the clinical phenotype with close follow-up. There have been reported cases of patients with TMA who developed immune complex–mediated MPGN, one of whom also carried a *DGKE* mutation.[4,5] The pathogenic and prognostic differences of this immune complex–mediated subgroup require further elucidation.

Regarding prognosis and treatment, most patients with *DGKE*-MPGN show some response to immunosuppressants. Nonetheless Azukaitis et al[4] reported a high prevalence of chronic proteinuria in *DGKE*-MPGN patients. Although statistically insignificant, a trend towards a lower 10-year renal survival was also observed (50% in *DGKE*-MPGN vs 89% in *DGKE*-HUS).[4] After initiation of CNI or mycophenolate mofetil, our patient showed a promising response and attained remission. At 5-year follow-up, he remained in remission with normal kidney function.

This is the first Chinese patient with *DGKE* nephropathy presenting as nephrotic syndrome with an MPGN picture. Of interest, our patient responded promptly to immunosuppressants, suggesting its potential role in this disease entity. This case highlights the importance of genetic testing in children with an atypical course of nephrotic syndrome. Further international collaborative studies are warranted to understand the pathogenesis and optimal management in this

patient population.

Author contributions

Concept or design: SHY Lau, EYH Chan.
Acquisition of data: All authors.
Analysis or interpretation of data: All authors.
Drafting of the manuscript: SHY Lau, EYH Chan.
Critical revision of the manuscript for important intellectual content: All authors.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

All authors have disclosed no conflicts of interest.

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Declaration

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Ethics approval

The patient was treated in accordance with the Declaration of Helsinki, with informed consent provided by the patient's mother for treatment, procedures, and publication.

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