Supplemental Appendix

The Phenotypic Spectrum of Nephropathies Associated with Mutations in Diacylglycerol Kinase Epsilon (DGKE)

Karolis Azukaitis[1], Eva Simkova[2], Mohammad Abdul Majid[2], Matthias Galiano[3], Kerstin Benz[3], Kerstin Amann[4], Clemens Bockmeyer [4], Radha Gajjar[5], Kevin E Meyers[5], Hae Il Cheong[6,7], Bärbel Lange-Sperandio[8], Therese Jungraithmayr[9], Véronique Frémeaux-Bacchi[10,11], Carsten Bergmann[12], Csaba Bereczki[13], Monika Miklaszewska[14], Dorottya Csuka[15], Zoltán Prohászka[15], Patrick Gipson[16], Matthew G. Sampson[16], Mathieu Lemaire[17,18,19*], Franz Schaefer[20*]

Affiliations:

[1]Clinic of Pediatrics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[2]Pediatric Nephrology Department, Dubai Hospital, Dubai, United Arab Emirates
[3]Department of Pediatrics and Adolescent Medicine, University of Erlangen-Nürnberg, Erlangen, Germany
[4]Institute of Pathology, Department of Nephropathology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
[5]Division of Nephrology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
[6]Department of Paediatrics, Seoul National University, Children's Hospital, Seoul, Korea
[7]Research Coordination Center for Rare Diseases, Seoul National University Hospital, Seoul, Korea
[8]Pediatric Nephrology Division, Department of Pediatrics, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University, Munich, Germany
[9]Pediatric Nephrology Center, Klinikum Memmingen, Memmingen, Germany
[10]Department of Immunology, Assistance Publique–Hôpitaux de Paris, Hôpital Européen Georges-Pompidou, Paris, France
[11]Centre de Recherche des Cordeliers, Unité Mixte de Recherche en Santé (UMR-S) 872, Paris, France.
[12]Center for Human Genetics, Bioscientia, Ingelheim, Germany.
[13]Department of Pediatrics, University of Szeged, Szeged, Hungary
[14]Department of Pediatric Nephrology, Jagiellonian University Medical College, Cracow, Poland
[15]3rd Department of Internal Medicine, Research Laboratory and George Füst Complement Diagnostic Laboratory, Semmelweis University, Budapest, Hungary
[16] Department of Pediatrics, Nephrology Division, University of Michigan School of Medicine, Ann Arbor, Michigan, USA
[17]Program in Cell Biology, The Hospital for Sick Children Research Institute, Toronto, Canada
[18]Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, Canada
[19]Department of Paediatrics, Division of Nephrology, The Hospital for Sick Children, Toronto, Canada.
[20]Division of Paediatric Nephrology, Centre for Paediatric and Adolescent Medicine, Heidelberg University Hospital, Heidelberg, Germany

* The authors contributed equally to this work.

Corresponding author:
Dr Karolis Azukaitis
Phone: +37065621642
Email: k.azukaitis@gmail.com
Address: Santariskiu str. 4, LT-08661 Vilnius, Lithuania

Contents

Detailed case reports of each newly identified patient with DGKE nephropathy

Kindred 1

Family 1 is a consanguineous family from the United Arab Emirates with three affected siblings out of seven. Of note, one of the affected children (patient #1.3) died in early childhood due to HUS, with no detailed history available.

Patient #1.1 presented with signs of viral infection, thrombotic microangiopathy (TMA) and acute kidney injury (AKI) at the age of eight months. He was noted to be hypertensive at the time. The patient received only symptomatic treatment and acute peritoneal dialysis (PD) was initiated for two weeks with gradual recovery of kidney function. The patient subsequently experienced two HUS relapses at one and two years of age, both of which resolved spontaneously. The second relapse was accompanied by nephrotic-range proteinuria and hematuria; serum complement C3 was within the normal range. The patient remained severely hypertensive despite aggressive antihypertensive therapy. Persistent severe hypertension resulted in left ventricular failure at age of 12 years. Over the next 10 years, recurrent episodes of nephrotic-range proteinuria were documented, accompanied by gradual deterioration of renal function leading to end-stage renal disease (ESRD) and start of hemodialysis (HD) was at the age of 16 years. Now 25 years old, this patient is still on HD.

Patient #1.2 first presented with HUS episode with hematuria, nephrotic-range proteinuria and hypertension at two years of age. He had a past history of suspected nephrotic syndrome at the age of 11 months. The patient was treated with plasma infusions (PI). His condition improved gradually with sufficient residual kidney function. He had four further documented relapses of HUS treated with PI leading to ESRD requiring PD for eight months at the age of seven years. A membranoproliferative glomerulonephritis (MPGN)-like disorder was suspected but parents refused kidney biopsy. Another HUS relapse occurred at the age of 10 years with signs of cardiac failure and pulmonary edema treated with PI and HD was initiated again. Three months later the patient was started on Eculizumab due to mild persistent hemolysis, which was discontinued after four doses with no signs of improvement. The patient remained severely hypertensive despite multiple antihypertensive agents with recurrent episodes of nephrotic-range proteinuria without signs of TMA. No decrease in C3 levels was ever noticed. The patient is now 14 years old and remains on HD. The option of a renal transplantation was declined by parents for both patients.

It is important to note that neither patient was tested for STEC because they did not have diarrhea on presentation. It is very unlikely that STEC is the cause for HUS when two siblings have multiple HUS recurrences that are staggered over time.

Kindred 2

Patient #2.1 presented at the age of three months with clinical signs of TMA, severe hypertension, seizures, normal serum C3 levels and AKI requiring dialysis for 10 days after an episode of acute gastroenteritis. He was the first child of healthy, unrelated parents. The patient received only

symptomatic treatment (including dialysis) and kidney function recovered spontaneously within two weeks. A second TMA episode occurred at eight months that was associated with mild AKI and nephrotic-range proteinuria. Kidney biopsy was performed and showed signs of active glomerular TMA and MPGN-like pattern (Figure 2). On that basis, steroid therapy was started (intravenous pulse methylprednisolone, followed by oral prednisone at 2 mg/kg/48 h). Renal function improved and steroids were continued and tapered down over one and a half years.

Two HUS episodes were recorded at age one year and three years both characterized by mild AKI, nephrotic-range proteinuria and hematuria. Renal function again improved over eight weeks. The last episode triggered another course of steroids that was stopped after one year. The disease was quiescent thereafter, except for persistent hypertension and proteinuria (subnephrotic and nephrotic). At the last follow-up at the age of 15, kidney function was normal with subnephrotic proteinuria and microhematuria while the patient was on enalapril and losartan.

His sister (patient #2.2) was diagnosed with TMA at the age of two months while recovering from a varicella zoster infection. She presented with severe hypertension, nephrotic-range proteinuria, hematuria, seizures and AKI requiring HD for five days. Kidney biopsy showed signs of glomerular TMA and acute tubular necrosis (ATN) (Supplementary Figure 2). She was initially treated with plasma exchange (2 sessions). Based on her brother's response to steroids, she was started on the same regimen. Kidney function improved over two weeks with persisting nephrotic-range proteinuria, hematuria and hypertension. She developed first HUS relapse with severe oligoanuric AKI related to an intercurrent infection at the age of four months. The patient was started on dialysis, plasma exchange (PE) sessions (10 in total) and steroids. Despite the therapy renal function deteriorated and failed to improve, therefore chronic PD was started.

Over the following years, there were five episodes of febrile seizures associated with viral infections and five episodes of aseptic meningitis (except for one that was caused by Picorna virus). None of these episodes were accompanied by HUS relapses. Laboratory investigations consistent with complement activation were only documented once: the patient was six at the time and on dialysis. The patient was successfully transplanted at the age of seven (deceased donor). The usual immunosuppression regimen was used except for the prescription of PE, which was performed immediately before and after allograft implantation. Now, nearly 3 years after renal transplantation, the patient has normal renal function with no signs of proteinuria and hematuria.

Of note, angiotensin II type 1 receptor antibodies were detected in both patients. On that basis, patient #2.2 was switched to losartan at the age six. Her brother #2.1 received losartan since the age of 6 years due to nephrotic-range proteinuria despite enalapril treatment.

It is also important to note that patient 2.2 had no diarrhea at disease onset and was not tested for STEC based on her brother's recurrent HUS.

Kindred 3

Patient #3.1 presented at four years with nephrotic syndrome, glomerular hematuria, hypertension, non-immune hemolytic anemia and increased D-dimers levels within two weeks of a viral infection. He was the first child of healthy, unrelated parents. Renal function was normal at the time. Kidney biopsy showed signs typical of TMA. Detailed investigations of the alternative complement system did not reveal any abnormalities, including anti-factor H antibodies. Shiga toxin-mediated HUS was ruled out and ADAMTS 13 levels were normal therefore the presumptive diagnosis of aHUS was made. The patient was treated with ramipril and low-molecular weight heparin that was discontinued after seven days once the D-dimers level normalized. On discharge, the patient was prescribed ramipril. While proteinuria improved over the next months, it remained in the nephrotic-range, therefore candesartan was added to his regimen. Now aged five years, the patient has not had another episode of TMA. He has normal kidney function with persistent proteinuria, hematuria and normal blood pressure while on candesartan and ramipril.

Kindred 4

Patient# 4.1 is a six month-old Korean male who presented with signs of viral infection accompanied by oliguria, hematuria but without proteinuria. Hypertension was present and laboratory investigations were consistent with TMA. He was the first child of healthy, unrelated parents. The patient was treated with seven sessions of PI. Although HD was required for two weeks, his renal function improved over two weeks. All of the six HUS relapses recorded in the next six years were preceded by viral infections except for the last one. HUS relapses were associated with nephrotic-range proteinuria and hematuria without significant renal function impairment. The patient was started on a tapering regimen of weekly plasma infusion after each episode (range five to 27 sessions per episodes; see Supplementary Figure 3 for details). Plasma C3 and C4 levels were tested at each TMA episode and were always normal. Now nine years old, he has normal renal function and blood pressure, with residual microhematuria and nephrotic proteinuria.

Kindred 5

Patient #5.1 presented at seven months of age with influenza infection and oliguria. Laboratory investigations revealed signs of TMA and increased serum creatinine. Detailed complement profiling revealed moderately low C3 (56mg/dL, normal range 78-169) and C4 levels (12.5mg/dL, normal range 16-45); high soluble membrane attack complex (362 ng/mL, normal range 27-44) and C3a (269.8 ng/mL, normal range 25-88.2) were also recorded. ADAMTS13 activity was normal. She was started on continuous venovenous hemodialfiltration for three days, before being transitioned to PD. Rapid recovery of renal function within two weeks led to discontinuation of PD after three days. The patient was also prescribed daily PE therapy (three sessions) before starting Eculizumab three days after admission.

She is maintained on amlodipine for persistent hypertension and on regular Eculizumab infusions every three weeks with stable and preserved renal function. The patient has had three documented viral illnesses since her initial presentation, each of which has been associated with

proteinuria and varying degrees of hematuria, but no signs of overt TMA. Each time, urinary sediments returned to normal within two weeks. At the age of two years, one week after her regular Eculizumab infusion, the patient developed HUS relapse associated with a viral illness. The relapse was characterized by AKI, proteinuria, hematuria and mild transient decrease in C3. CH50% test that was taken immediately after her admission showed sufficient complement system suppression (6, normal range – 101-350). The patient was started on HD and treated with PE (nine sessions), PI (one session) and intensified Eculizumab regimen (weekly infusions, six in total). The treatment led to renal function recovery and HD withdrawal after 13 sessions. The clinical course of HUS relapse on Eculizumab maintenance therapy is depicted in Supplementary Figure 5. Two months after the relapse the patient is maintained on two-weekly Eculizumab infusions with normal renal function and traces of protein on her urinary sediment.

Kindred 6

Patient #6.1 presented to the hospital with periorbital edema, nephrotic-range proteinuria, hematuria and signs of TMA following a febrile upper respiratory tract infection at the age of two years. She was a previously healthy child of healthy unrelated parents. Her kidney function was normal and she was non-hypertensive. ADAMTS13 activity was only mildly decreased and she had no signs of complement activation with negative anti-factor H antibodies. Kidney biopsy was consistent with a diagnosis of TMA and mild tubulopathy. Treatment with steroids pulse and PI was initiated, which later was changed to PE (five sessions in total). Worsening of TMA and kidney function was observed after initiation of PE and the patient was switched to Eculizumab treatment which was discontinued after two doses because of lack of effect (Supplementary Figure 4). Patient's kidney function decreased, she became hypertensive and at two months since the onset of the disease the patient was started on HD with persistent proteinuria and hematuria and continuous signs of TMA with red blood cells transfusions dependency. Renal function gradually improved leading to HD withdrawal after one month. At last follow-up 10 months since disease onset the patient is on ACE inhibitor therapy, hypertensive with normal GFR, mild proteinuria and hematuria. Signs of complement activation (mild decrease of C3 and an increase of terminal complement complex) were observed during disease progression.

Kindred 7

Patient #7.1, a child of healthy unrelated parents, presented with signs of TMA and anuric AKI requiring dialysis after an episode of non-bloody diarrhea at the age of five months. She was severely hypertensive requiring five antihypertensive medications to achieve blood pressure control. Treatment with PI was initiated leading to improvement of kidney function and dialysis was discontinued after 16 days. Decreased C3 and C4 levels were only observed while on dialysis. Urinalysis showed nephrotic proteinuria and hematuria. Despite improvement of kidney function the patient remained severely hypertensive, requiring multiple antihypertensive regimen. The patient was put on ACE inhibitors with resolution of proteinuria and mild persisting hematuria. A relapse occurred after an upper respiratory tract infection at the age of one year that was treated with PI infusions with moderate response. During viral infections the patient two times presented with transient proteinuria up to 2.5 g/L that lasted for a few days and decreased spontaneously to trace level after the infection had terminated. At last follow-

up, the patient who was at the time two years, had normal kidney function and no proteinuria, but mild hematuria and hypertension persists requiring four antihypertensive medications in maximum doses.

Kindred 8

Patient #8.1, a 2.5 y boy with no family history of kidney disease presented to his pediatrician after a few days of vomiting and non-bloody diarrhea. IV fluids were infused because of dehydration. While hydration status improved post-infusion, the child was noted to have peripheral edema, with a weight gain of 1.5-2 kg. Laboratory investigations done at that time revealed hypoalbuminemia (serum albumin 1.7 g/dL) and albuminuria, consistent with a diagnosis of nephrotic syndrome. He was also noted to be hypertensive (110/54 mm Hg). On presentation, hemoglobin was 97 g/L and platelets were 73, without shistocytes on blood smear. He was transferred to a tertiary care institution for further care. Two anti-hypertensive medications were started during the following week to control the blood pressure. Over the following week, laboratory studies showed microangiopathic hemolytic anemia, thrombocytopenia, and hematuria and later AKI.

Of note, this patient was never tested for STEC because on initial presentation, he had mild non-bloody diarrhea that was accompanied by nephrotic syndrome, not thrombotic microangiopathy. Testing of stool samples was not possible once the findings of TMA started to become obvious after admission.

Past medical history was unremarkable except for a prior diagnosis of transient erythroblastopenia of childhood (TEC). The pediatrician had found that hemoglobin was 7.0 g/dL (normal 10.5-13.5) with high hemoglobin F and reticulocytosis a few months before the first visit to hematology. Laboratory investigations to document the presence of hemolysis or kidney dysfunction were not done. Iron supplementation was initiated. When the patient was seen by the hematologist at age 19 months, hemoglobin had normalized (11.0 g/dL), DAT was negative, and iron stores were normal; renal function was also normal. Because the hemoglobin was stable 1 month later (12.0 g.dL), a diagnosis of TEC was posed and iron supplementation was discontinued.

Renal biopsy done during the admission was diagnosed as MPGN with extensive immune deposits and fibrin deposition. The additional comments are however important since they illustrate well the key findings:

> "This is a chronic glomerulonephritis characterized by extensive subendothelial and mesangial electron dense deposits which appear to have recruited inflammatory cells, stimulated the proliferation of the mesangial cells and elicited a sclerotic reaction with increased mesangial matrix and duplication of the GBM. Immune reactants include IgG (2+), IgA (2+), IgM (3+) and classical pathway complement components (C3, 2+; C1q, 2+). In addition, there appears to be significant endothelial injury manifested by swelling, vacuolization and myelin figures, associated with fibrin in the lumen and subjacent to the endothelial cells. Focal and segmental fibrinogen staining was observed within vascular lumens of capillary loops of glomeruli. This is most likely secondary to the patient's profound hypertension."

AKI resolved over several week and renal replacement therapy was not required. For nephrotic-range proteinuria, trials of intravenous steroids, cyclosporine, and cyclophosphamide were not successful. Alongside oral steroids, he underwent treatment with plasmapheresis and vincristine, which resulted in improvement in hypoalbuminemia, and then normalization of complement levels within weeks. Hypertension and proteinuria improved over several months. For many years, he was maintained on only ACE inhibitor which controlled proteinuria, but he did have persistent hematuria. There were no HUS recurrences after initial presentation. When the patient was 17, proteinuria worsened despite optimization of ACE inhibitor therapy; renal function, complement levels, hemoglobin, and platelet counts remained normal. Renal biopsy showed signs of acute and chronic endothelial injury with tubular atrophy and interstitial fibrosis that were interpreted as consistent with aHUS.

A repeat kidney biopsy was done two years later (19 y) because of worsening proteinuria and progression of kidney dysfunction to CKD stage 2 (creatinine 1.3 mg/dL)**.** The biopsy was deemed to be unchanged when compared to the one done two years earlier. The salient findings on light microscopy were diffusely thickened capillary walls with duplication of basement membranes and numerous small lacunae that do not stain strongly with silver (consistent with foreign proteinaceous deposits within the capillary wall); segmentally swollen endothelial cells; diffusely expanded mesangium matrix without hypercellularity. Immunofluorescence studies revealed granular IgM staining (1-2+) in capillary loops, small arterioles and mesangial deposits; C3 staining in small arterioles (2+) and capillary loops (trace); trace C4 and C1q staining in capillary loop deposits; trace fibrinogen staining along glomerular capillary surfaces and tubular brush borders. All other investigations were negative (IgG, IgA, IgM, albumin, and kappa- and lambda light chains). The electron microscopy investigations showed extensive foot process effacement; marked wrinkling/distortion of glomerular capillaries; markedly thickened capillary wall with multi-lamination of the GBM; swollen endothelial cells with occasional complete obliteration of vascular lumen; prominent subendothelial basement membrane, especially where it overlies the mesangium; modest expansion of the mesangium, with mild increase in cell numbers.

Of note, C3NeF levels were measured in the normal range when the patient was 19 years. This result must be interpreted with caution since renal disease was already established at that time.

Supplemental Table 1. Assessment of the pathogenic potential of the two missense DGKE mutations

Prediction Softwares[2-7]	**Novel DGKE missense mutations**		**Deleterious thresholds**
	p.K109E	p.C167W	
Polyphen-2	Probably damaging Score: 1.0	Probably damaging Score: 1.0	Score > 0.5
SIFT	Damaging Score: 0.003	Damaging Score: 0.000	Score > 0.05
PROVEAN	Deleterious Score: -3.10	Deleterious Score: -9.18	Score > -2.5
Mutation assessor	Functional impact: Medium Score: 3.03	Functional impact: High Score: 3.93	Score > 0.65
SNAP2	Effect on protein function (non-neutral) Score: 78	Effect on protein function (non-neutral) Score: 85	Effect: 0 to100 Neutral: -100 to 0
PANTHER	Probably damaging Preservation time: 1037	Probably damaging Preservation time: 1629	Probably damaging > 450 Possibly damaging 200-450 Probably benign < 200
CONDEL	Damaging Score: 0.60	Damaging Score: 0.78	Score > 0.49

Supplemental Table 2. Description of the two cohorts used to estimate DGKE prevalence in pediatric aHUS patients

	Heidelberg cohort (n=96)[a]	**Seoul cohort (n=51)[b]**
Kindreds with DGKE nephropathy	Kindred #1 Kindred #2 Kindred #9 (already described[8])	Kindred #4
# of patients with genetic AP abnormalities	29/96 (30.2 %)	10/51 (19.6 %)
# of anti-CFH positive patients	Not screened	15/51 (29.4 %)
# of unrelated patients with DGKE mutations	3/63[c]	1/51
Overall prevalence	3.1%	2.0%
Prevalence in patients without genetic AP abnormalities	4.7 %	2.4 %
Prevalence in patients without genetic and immunologic AP abnormalities	NA	3.8 %

[a] Unrelated children of various age, sex and ethnicity with aHUS

[b] South Korean cohort of children with aHUS (detailed description of the cohort in reference 1)

[c] Number of patients screened for DGKE mutations (all without genetic AP abnormalities).

Supplemental Table 3. Demographic and clinical characteristics for all previously reported patients with DGKE mutations

Index #	Kindred #	Original ID	Age at Dx (yr)	Gender	Origin	Familial variables	DGKE mutations	Complement activation	Clinical diagnosis	Reference
1	1	1-3	0.7	M	Europe	NC	p.Trp322*	No	TMA	8
2	1	1-4	0.3	F	Europe	NC	p.Trp322*	No	TMA	8
3	2	2-5	0.6	F	North Africa	NC	p.Arg63Pro p.Val163Serfs*3	No	TMA	8
4	2	2-7	0.3	F	North Africa	NC	p.Arg63Pro p.Val163Serfs*3	No	TMA	8
5	3	3-3	0.5	F	Europe	NC	p.Trp322* p.Ser11*	No	TMA	8
6	4	4-1	0.3	F	Europe	NC	p.Trp322*	No	TMA	8
7	5	5-3	0.6	M	Europe	NC	p.Trp322* p.Trp158Leufs*8	No	TMA	8
8	6	6-3	0.5	F	Europe	Csg	p.Gln334*	No	TMA	8
9	7	7-3	0.9	M	Europe	Csg	p.IVS5-1	No	TMA	8
10	8	8-3	0.7	M	Europe	Csg	p.Trp322*	No	TMA	8
11	9	9-3	0.3	M	Middle East	Csg	p.Arg273Pro	No	TMA	8
12	9	9-4	0.8	M	Middle East	Csg	p.Arg273Pro	No	TMA	8
13	9	9-6	0.3	F	Middle East	Csg	p.Arg273Pro	No	TMA	8
14	10	UT-062 V-2	5	M	Middle East	Csg	p.Gln43*	No	MPGN	9
15	10	UT-062 V-3	2	M	Middle East	Csg	p.Gln43*	No	MPGN	9
16	10	UT-062 V-4	4	M	Middle East	Csg	p.Gln43*	No	MPGN	9
17	10	UT-062 V-6	0.8	M	Middle East	Csg	p.Gln43*	No	MPGN	9
18	11	HU-314 IV-1	17	F	Middle East	Csg	p.Thr204Glnfs*6	No	MPGN	9
19	11	HU-314 IV-2	8	F	Middle East	Csg	p.Thr204Glnfs*6	No	MPGN	9
20	12	HU-500 V-1	1.5	F	Middle East	Csg	p.IVS5-2[a]	No	MPGN	9

21	12	HU-500 V-2	1.5	M	Middle East	Csg	p. IVS5-2[a]	No	MPGN	9
22	12	HU-500 V-3	1.5	M	Middle East	Csg	p. IVS5-2[a]	No	MPGN	9
23	13	58	0.8	F	Europe	Csg	p.Lys101*	Yes	TMA	10
24	13	59	0.7	M	Europe	Csg	p.Lys101*	Yes	TMA	10
25	14	HUS299	1.1	M	North Africa	NC	p.His536Glnfs*16	No	TMA	11
26	15	HUS39	0.3	M	Europe	NC	p.Trp322* p.Pro498Arg	No	TMA	11
27	15	HUS40	0.6	F	Europe	NC	p.Trp322* p.Pro498Arg	Yes	TMA	11
28	16	HUS272	0.7	F	Europe	NC	p.Gln248His p.Gly484Glyfs*10	Yes	TMA	11
29	17	xx	0.3	M	Asia	NC	c.1213–2A>G p.Leu24Cysfs*145	Yes	TMA	12
30	18	452	0.8	F	Europe	Csg	c.888+40A>G	Yes	TMA	13
31	18	1200	0.4	M	Europe	Csg	c.888+40A>G	Yes	TMA	13
32	19	II-1	0.8	M	Europe	NC	c.888+40A>G p.Trp322*	No	TMA	13
33	19	II-2	0.6	M	Europe	NC	c.888+40A>G p.Trp322*	No	TMA	13
34	19	II-4	0.4	F	Europe	NC	c.888+40A>G p.Trp322*	No	TMA	13

Note: A total of four siblings of reported patients died from HUS in early childhood with no genetic data (one sibling of a patient reported by Lemaire et al.[7]; two from the kindred reported by Westland et al.[9]; one from kindred I described in this article)

[a]While reviewing the data, we noticed a small mistake in the description of the mutation for kindred HU-500. Please see Supplemental Figure 6 for details.

Supplemental Table 4. Clinical and laboratory data for the first episode for all previously reported DGKE patients

Index #	Kindred #	Original ID	Hgb nadir (g/dL)	Evidence of TMA	Platelet nadir (10^3/μL)	sCr peak (mg/dL)	Proteinuria	Hematuria	Hypertension	Trigger	Dialysis	Other treatments	Recovery of renal function
1	1	1-3	7.2	Yes	50	2.84	n/a	n/a	n/a	Unknown	Yes	None	Yes
2	1	1-4	5.7	Yes	36	1.89	n/a	n/a	n/a	Unknown	Yes	PE	Yes
3	2	2-5	7.3	Yes	35	5.32	n/a	n/a	n/a	Unknown	No	PI, IVIG	Yes
4	2	2-7	6.8	Yes	168	1.35	n/a	n/a	n/a	Unknown	No	None	Yes
5	3	3-3	8.4	Yes	132	0.62	n/a	n/a	n/a	Unknown	No	None	Yes
6	4	4-1	3.7	Yes	390	8.51	n/a	n/a	n/a	Unknown	Yes	PI	Yes
7	5	5-3	7.1	Yes	88	5.09	n/a	n/a	n/a	Unknown	Yes	None	Yes
8	6	6-3	9	Yes	99	2.52	n/a	n/a	n/a	Unknown	Yes	PE	Yes
9	7	7-3	4.9	Yes	125	6.79	n/a	n/a	n/a	Unknown	Yes	PE	Yes
10	8	8-3	5	Yes	214	7.19	n/a	n/a	n/a	Unknown	Yes	PE	No
11	9	9-3	6.4	Yes	33	4.21	n/a	n/a	n/a	Unknown	Yes	PI	Yes
12	9	9-4	8.2	nd	57	0.6	n/a	n/a	n/a	Unknown	No	None	Yes
13	9	9-6	7.3	Yes	32	2.21	n/a	n/a	n/a	Unknown	Yes	PI	Yes
14	10	UT-062 V-2	n/a	No	n/a	3	Yes, NR	n/a	n/a	Unknown	n/a	Steroids, cyclophosphamide/CsA, ACEI	No
15	10	UT-062 V-3	n/a	No	n/a	0.6	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide/CsA, ACEI	No
16	10	UT-062 V-4	n/a	No	n/a	0.6	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide/CsA, ACEI	No
17	10	UT-062 V-6	n/a	No	n/a	0.6	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide/CsA, ACEI	No
18	11	HU-314 IV-1	n/a	No	n/a	1.9	Yes, NR	n/a	n/a	Unknown	No	Steroids, CsA	No
19	11	HU-314 IV-2	n/a	No	n/a	0.68	Yes	n/a	n/a	Unknown	No	Steroids, CsA, ACEI	Yes
20	12	HU-500 V-1	n/a	No	n/a	0.4	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide	Yes
21	12	HU-500 V-2	n/a	No	n/a	0.77	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide	Yes

22	12	HU-500 V-3	n/a	No	n/a	0.34	Yes, NR	n/a	n/a	Unknown	No	Steroids, cyclophosphamide	Yes
23	13	58	n/a	Yes	64	1.5	Yes	Yes	n/a	Unknown	n/a	PI	Yes
24	13	59	n/a	Yes	30	11.4	Yes	Yes	n/a	Yes (viral)	n/a	PI	Yes
25	14	HUS299	7.7	Yes	17	7.7	Yes, NR	n/a	Yes	Yes (gastroenteritis)	Yes	None	Yes
26	15	HUS39	n/a	Yes	n/a	2.8	Yes, NR	n/a	n/a	Unknown	Yes	PI	Yes
27	15	HUS40	n/a	Yes	n/a	4	Yes, NR	n/a	n/a	Unknown	Yes	None	Yes
28	16	HUS272	13.4	Yes	129	0.6	Yes, NR	Yes	Yes	Yes (URTI)	No	ACEI	Yes
29	17	xx	8.9	Yes	41	1.95	n/a	n/a	Yes	Yes (gastroenteritis)	Yes	PI, PE, Eculizumab	Yes
30	18	452	10.7	Yes	63	4.17	Yes	Yes	Yes	Unknown	No	None	Yes
31	18	1200	10.9	Yes	96	0.7	Yes	Yes	Yes	Unknown	No	None	Yes
32	19	II-1	9.8	Yes	85	0.7	Yes, NR	Yes	n/a	Unknown	No	None	Yes
33	19	II-2	8	Yes	34	7.3	n/a	n/a	n/a	Unknown	Yes	None	Yes
34	19	II-4	8.3	Yes	122	5.3	n/a	n/a	n/a	Unknown	Yes	None	Yes

Supplemental Table 5. Long-term outcomes and pathological data for all previously reported patients with DGKE mutations

IDs			TMA relapse(s)						Long-term outcomes					
Index #	**Kindred #**	**Original ID**	**#**	**Trigger**	**Proteinuria**	**Hematuria**	**Acute treatment**	**Maintenance therapy**	**Age at last follow-up (yrs)**	**Current CKD stage**	**Age at ESRD (yrs)**	**Renal Tx**	**Proteinuria**	**Hematuria**
1	1	1-3	0	-	-	-	-	Eculizumab	11	CKD1	-	No	Yes	Yes
2	1	1-4	1	Unknown	n/a	n/a	PE, Eculizumab	Eculizumab	4	CKD1	-	No	Yes	Yes
3	2	2-5	1	Unknown	n/a	n/a	None	None	11	Tx	11	Yes	Yes, NR	Yes
4	2	2-7	4	Unknown	n/a	n/a	PE	Eculizumab	21	CKD4	-	No	Yes	Yes
5	3	3-3	1	Unknown	n/a	n/a	Dialysis, PE, PI, CsA, IVIG	None	21	CKD4	-	No	Yes	Yes
6	4	4-1	0	-	-	-	-	None	18	Tx	18	Yes	Yes	Yes
7	5	5-3	1	Unknown	n/a	n/a	CsA, MMF	None	11	CKD1	-	No	Yes, NR	Yes
8	6	6-3	3	Unknown	n/a	n/a	PE, Eculizumab	Eculizumab	4	CKD1	-	No	Yes	Yes
9	7	7-3	1	Unknown	n/a	n/a	PE, Eculizumab	Eculizumab	3	CKD1	-	No	Yes	Yes
10	8	8-3	0	Unknown	n/a	n/a	-	None	1	Tx	1	Yes	Yes	Yes
11	9	9-3	2	Unknown	n/a	n/a	CsA, MMF	None	13	HD	13	No	Yes, NR	Yes
12	9	9-4	2	Unknown	n/a	n/a	PI	PI, Eculizumab	8	CKD1	-	No	Yes	Yes
13	9	9-6	5	Unknown	n/a	n/a	PI	PI, Eculizumab	5	CKD1	-	No	Yes	Yes
14	10	UT-062 V-2	0	-	-	-	-	-	20	Tx (last sCr 1.46)	8 yrs	Yes	Yes	n/a
15	10	UT-062 V-3	0	-	-	-	-	-	4	Died from meningitis	-	No	n/a	n/a

16	10	UT-062 V-4	0	-	-	-	-	-	30	Last sCr 2	-	No	Yes, NR	n/a
17	10	UT-062 V-6	0	-	-	-	-	-	19	CKD5	19 yrs	No	Yes, NR	n/a
18	11	HU-314 IV-1	0	-	-	-	-	-	23	CKD5	23 yrs	No	Yes, NR	n/a
19	11	HU-314 IV-2	0	-	-	-	-	-	19	CKD1	-	No	Yes, trace	n/a
20	12	HU-500 V-1	0	-	-	-	-	-	12	CKD1	-	No	Yes	n/a
21	12	HU-500 V-2	0	-	-	-	-	-	2	CKD1	-	No	Yes, NR	n/a
22	12	HU-500 V-3	0	-	-	-	-	-	2	CKD1	-	No	Yes, NR	n/a
23	13	58	0	-	-	-	-	PI	5	CKD1	-	No	Yes	Yes
24	13	59	0	-	-	-	-	PI	3	CKD1	-	No	No	Yes
25	14	HUS299	0	-	-	-	-	-	4	CKD1	-	No	Yes	No
26	15	HUS39	1	Unknown	n/a	n/a	PI	PI	11	CKD3	-	No	No	No
27	15	HUS40	3	Yes (URTI)	n/a	n/a	Dialysis, PI	PI	17	CKD3	-	No	Yes	No
28	16	HUS272	>3	Yes (vaccination, URTI)	Yes	Yes	PI	PI, Eculizumab	4	CKD1	-	No	Yes, NR	Yes
29	17	xx	0	-	-	-	-	Eculizumab	1.5	CKD2	-	No	No	n/a
30	18	452	1-3/yr	Yes (infections)	Yes, NR	Yes	PI/PE	PI	13	CKD1	-	No	Yes	No
31	18	1200	1-3/yr	Yes (infections)	Yes, NR	Yes	PI/PE	PI	10	CKD1	-	No	Yes	No
32	19	II-1	5	Unknown	Yes, NR	n/a	Dialysis	None	13	CKD1	-	No	Yes	Yes
33	19	II-2	2	Unknown	n/a	n/a	None	None	10	CKD1	-	No	Yes, NR	Yes
34	19	II-4	3	Unknown	Yes, NR	n/a	None	None	4	CKD1	-	No	Yes	Yes

Supplemental Table 6. Pathological data for all previously reported patients with DGKE mutations who had a renal biopsy

Index #	Kindred #	Original ID	Number of renal biopsies done	Age when biopsy done (yr)	Main findings and diagnosis
1	1	1-3	2	2; 9	TMA
2	1	1-4	ND	-	_
3	2	2-5	2	1; 3	TMA
4	2	2-7	2	1.5; 21	TMA; Fibrosis
5	3	3-3	3	0.7; 1; 5	TMA
6	4	4-1	3	1; 2; 4	TMA
7	5	5-3	2	1; 5	TMA
8	6	6-3	3	1; 3; 4	TMA
9	7	7-3	1	2	TMA
10	8	8-3	1	1	TMA, global renal cortical ischemia and malignant nephroangiosclerosis noted in both kidneys
11	9	9-3	1	7	TMA
12	9	9-4	ND	-	_
13	9	9-6	ND	-	_
14	10	UT-062 V-2	2	n/a	MPGN (native kidney); De novo stage I membranous nephropathy in transplanted kidney
15	10	UT-062 V-3	1	2	MPGN
16	10	UT-062 V-4	1	4	MPGN
17	10	UT-062 V-6	1	0.8	MPGN
18	11	HU-314 IV-1	1	17	MPGN, secondary focal and segmental sclerotic glomeruli
19	11	HU-314 IV-2	1	8	MPGN
20	12	HU-500 V-1	1	1.5	MPGN
21	12	HU-500 V-2	1	1.5	MPGN
22	12	HU-500 V-3	1	1.5	MPGN
23	13	58	1	1	TMA
24	13	59	ND	-	_

25	14	HUS299	ND	-	_
26	15	HUS39	ND	-	_
27	15	HUS40	1	1	TMA
28	16	HUS272	1	0.7	TMA
29	17	xx	1	0.75	TMA
30	18	452	n/a	-	_
31	18	1200	n/a	-	_
32	19	II-1	n/a	-	_
33	19	II-2	n/a	-	_
34	19	II-4	n/a	-	_

Supplemental Table 7. Comparison of various clinical variables between patients with different genotypes

	Genotype		p value
Phenotypic feature	Expected loss-of-function[a] (n=33)	Others (n=11)	
Age (years)	0.8 (1)	0.6 (0.4)	0.0788
Serum creatinine (mg/dL)	1.9 (3.49)	2.8 (2.88)	0.6447
Relapses per year[b]	0.07 (0.57)	0.19 (0.52)	0.1701
Proteinuria at onset	21/21[c]	5/5	-
Proteinuria at follow-up	27/32	10/11	0.5895
ESRD development	6/33	4/11	0.2127
Complement activation	8/33	2/11	0.6779

Continuous data presented as median (interquartile range)

[a] Expected loss-of-function" defined as two alleles with either splice site, frameshift and/or non-sense mutations

[b] Relapse rate calculated for number of follow-up years not on RRT. Only DGKE HUS patients were included in the calculation.

[c] Only patients without missing data for each question were included in the analysis.

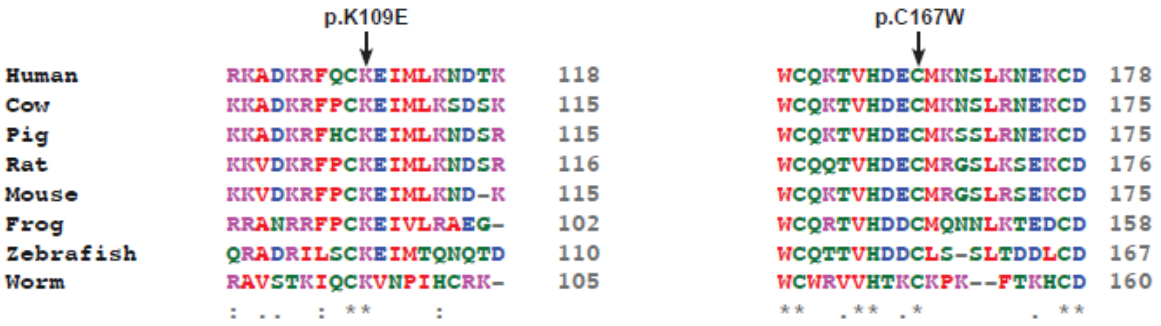
Supplemental Table 8. Genetic and immunologic analysis for each family

Family	Mode of variant identification	Complement AP testing		STEC testing
		Genes tested	Anti-CFH antibodies	
Family I	Retrospective (cohort screening)[a]	CFH, CFB, CFI, MCP, C3, CFHR1, CFHR3	Not tested	Not tested
Family II	Retrospective (cohort screening)[a]	CFH, CFB, CFI, MCP, C3, CFHR1, CFHR3	Negative	*Only for patient 2.1:* Culture ST test Anti-LPS antibodies
Family III	Diagnostic work-up	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3	Negative	EHEC PCR
Family IV	Retrospective (cohort screening)[a]	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3	Negative	ST test
Family V	Diagnostic work-up	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3	Negative	Culture ST test
Family VI	Diagnostic work-up	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3	Negative	Culture ST test
Family VII	Retrospective	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3, CFHR5	Negative	Culture
Family VIII	Retrospective	CFH, CFB, CFI, MCP, THBD, C3, CFHR1, CFHR3	Negative	Not tested

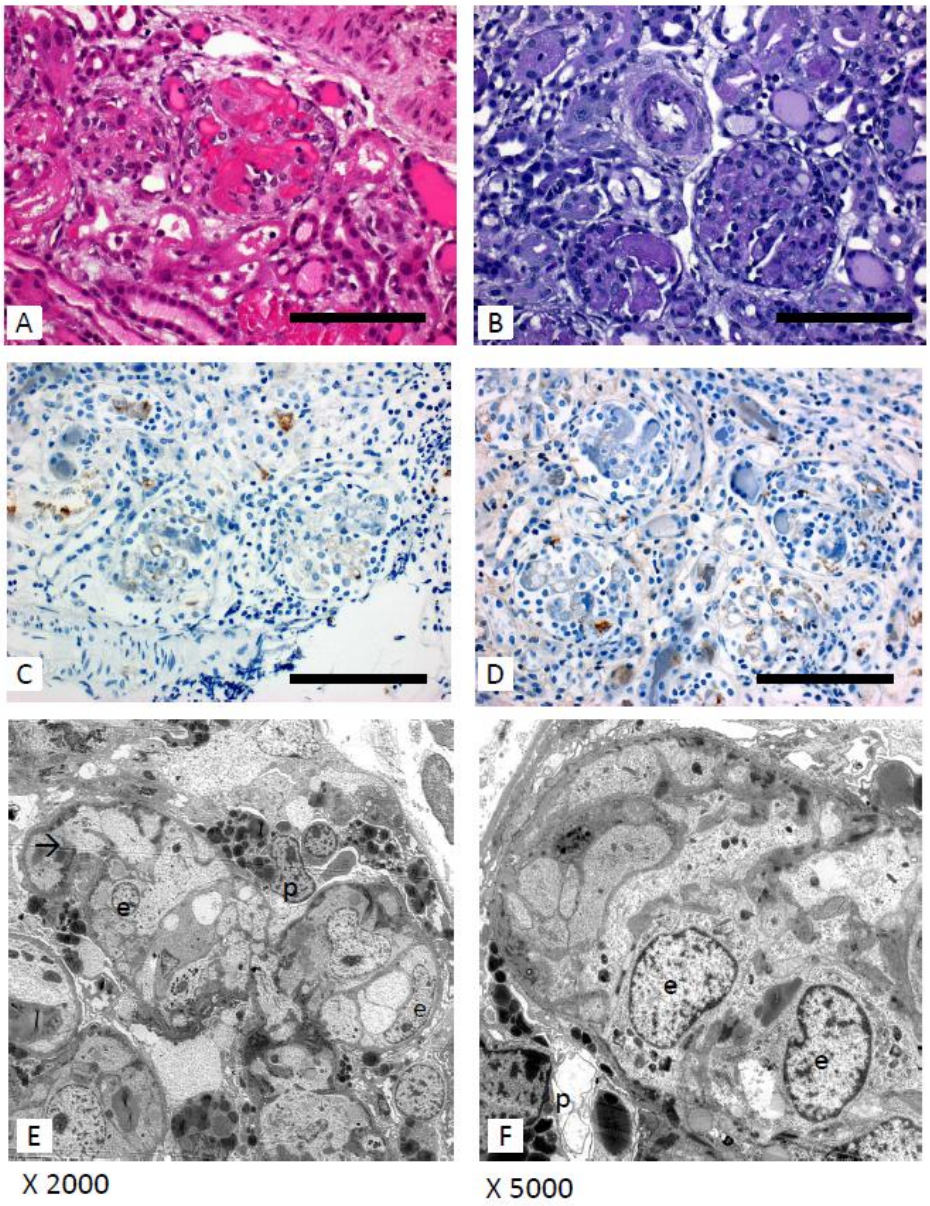
EHEC, Enterohemorrhagic E. coli; PCR, polymerase chain reaction; STEC, Shiga-toxin producing E. coli; ST, Shiga toxin.

[a]Descriptions of these cohorts are provided in Supplementary Table 2.

Supplemental Figure 1. Amino acid conservation at both loci harboring missense DGKE mutations

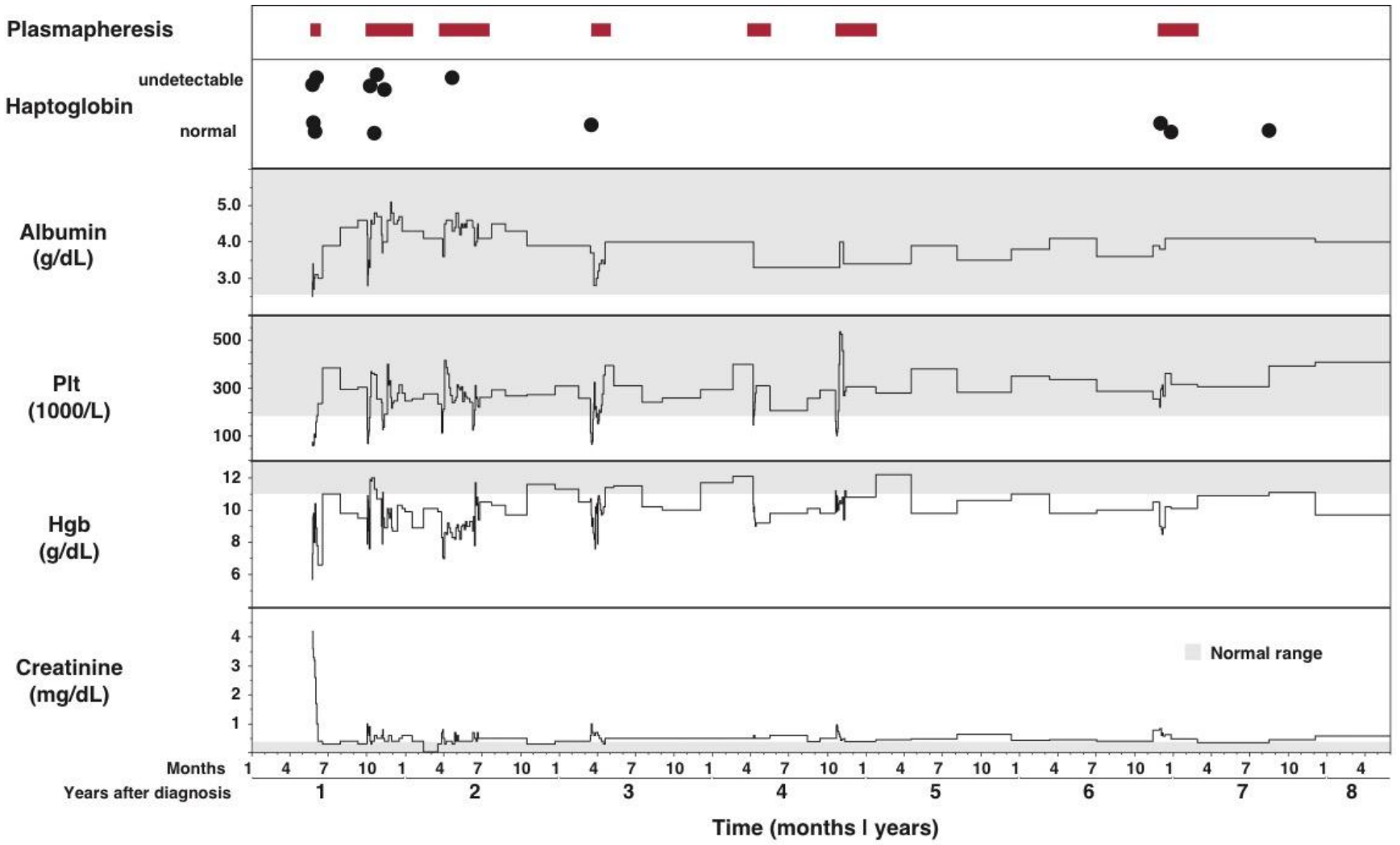


Supplemental Figure 2. Renal histology of patient #2.2

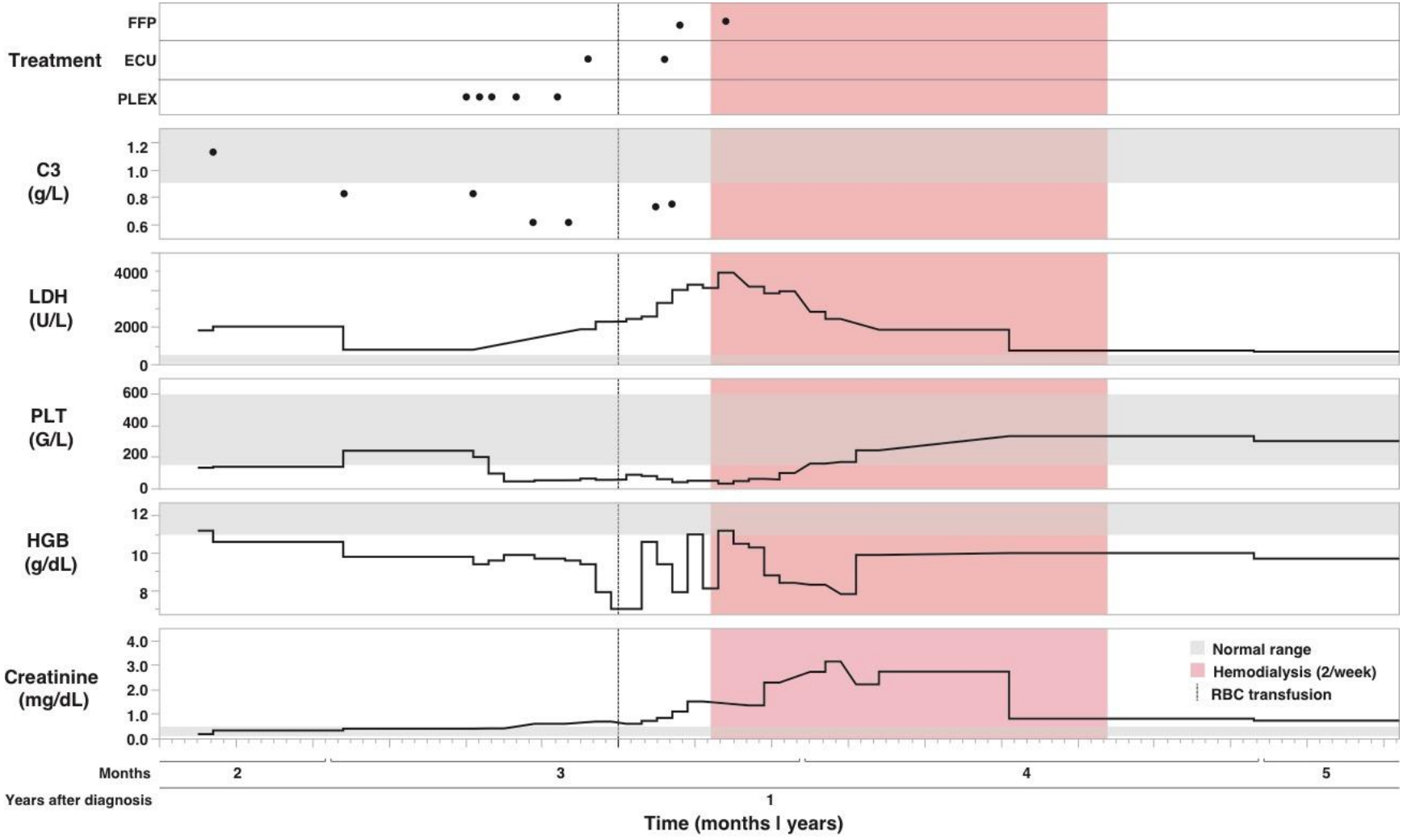


A,B: Light microscopical changes of affected glomeruli and surrounding tubulointerstitium with active glomerular thrombotic microangiopathy, hyperemia of glomeruli, partial collapse of the capillary tuft, thickening and wrinkling of glomerular basement membrane and swelling of endothelial cells. In addition, tubulointerstitial edema and acute tubular damage can be seen. No tubular atrophy or interstitial fibrosis. No inflammation. A: HE stain, magnification x 40, B: PAS stain, magnification x 40. **C,D:** Immunohistochemistry using antibodies against IgG and C3c shows no specific glomerular staining (magnification x 40). **E,F:** Electron microscopy at various magnifications (x2000, x5000) showing marked endothelial cell swelling (e) with subendothelial cleft formation (→), capillary lumen obliteration, adhesion of inflammatory cells (not shown) and marked swelling of podocytes (p) with foot process effacement. Glomerular basement membrane is intact. No specific osmiophilic deposits, no fibrils.

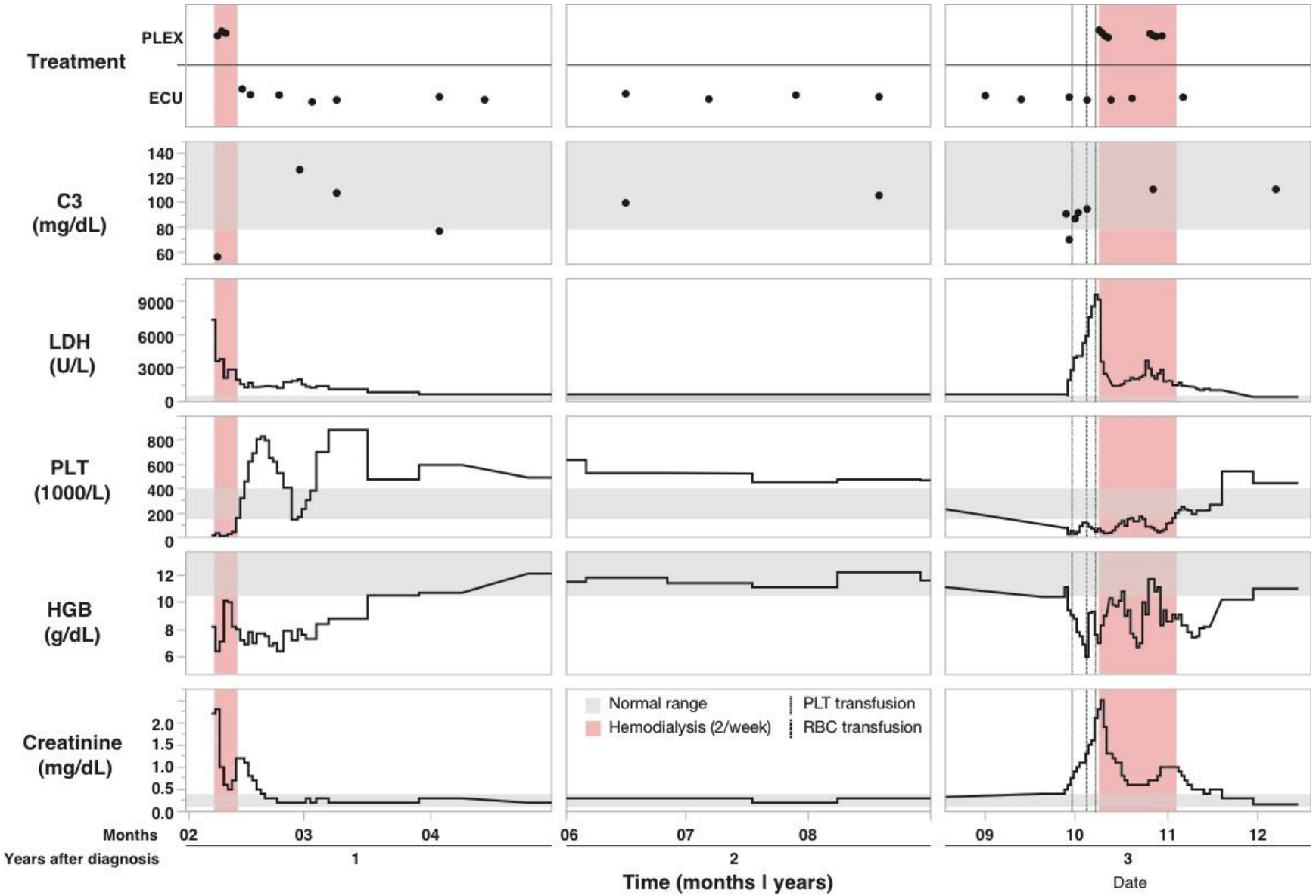
Supplemental Figure 3. Clinical course of patient #4.1 on intermittent plasmapheresis.



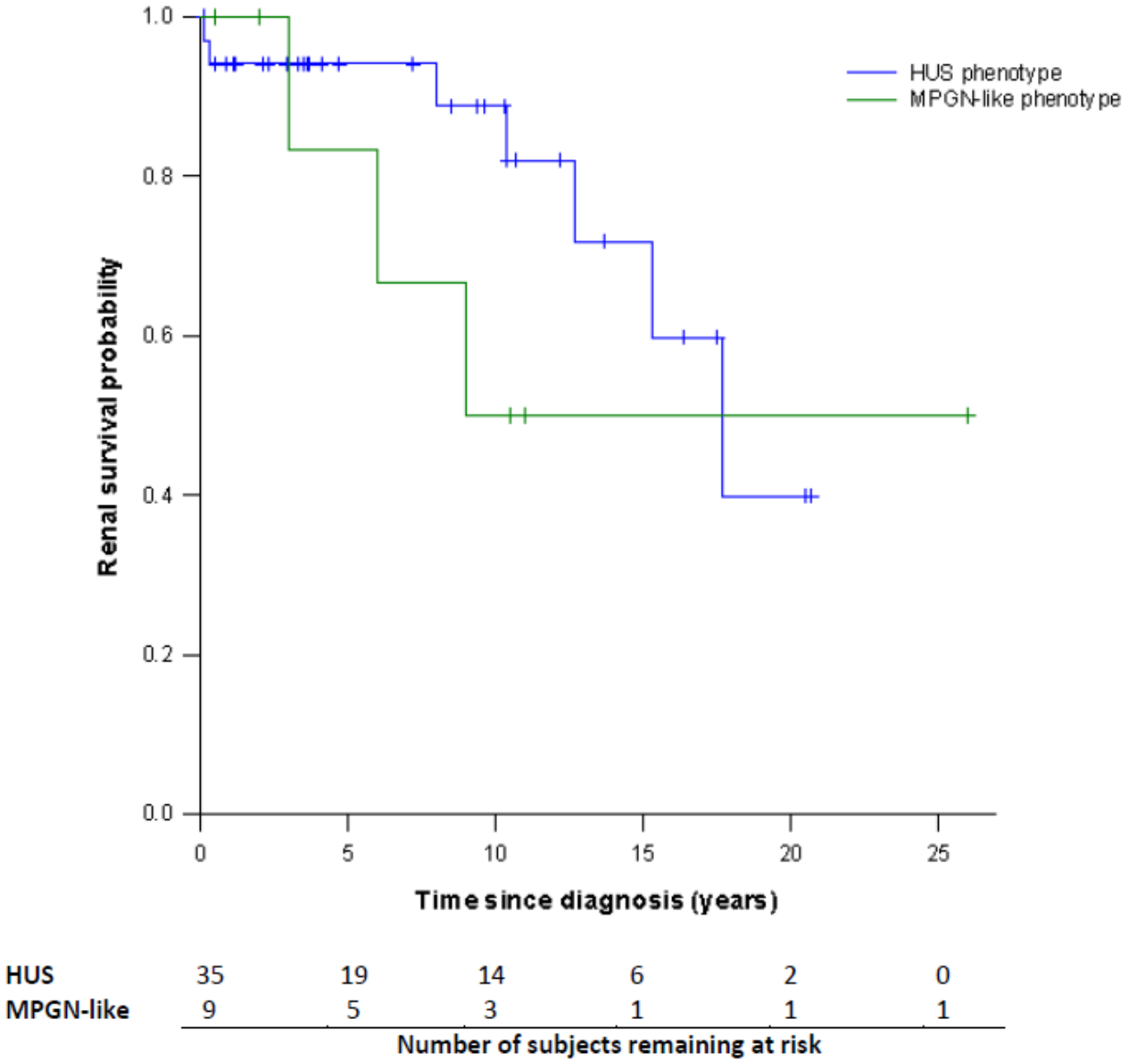
Supplemental Figure 4. Clinical course of patient #6.1 on Eculizumab therapy**.**



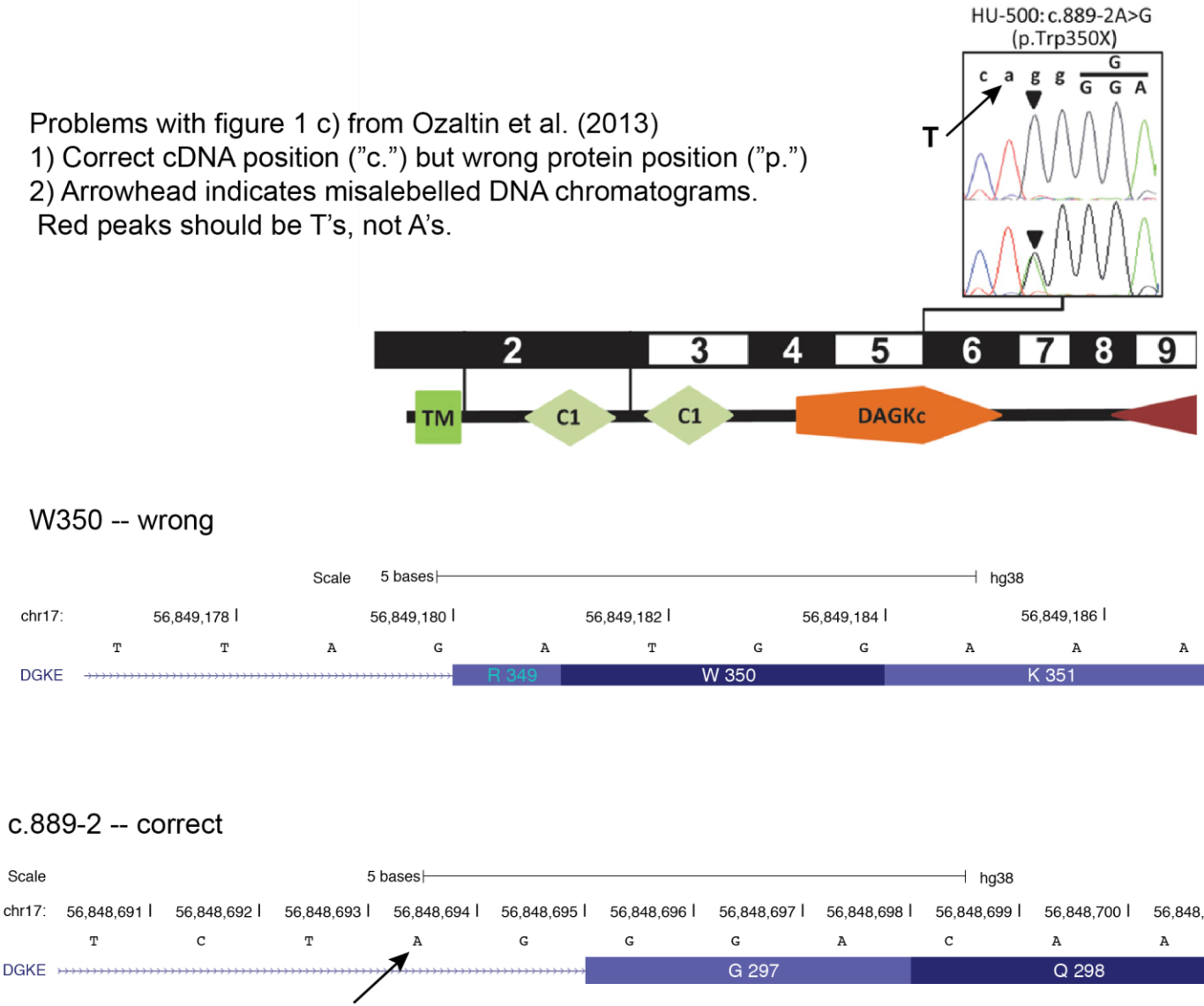
Supplemental Figure 5. Clinical course of patient #5.1 on Eculizumab therapy.



Supplemental Figure 6. Kaplan-Meier renal survival curve, defined as progression to ESRD, for HUS phenotype an MPGN-like phenotype patients. The numbers below X-axis show number of subjects remaining at risk at five year intervals.



Supplemental Figure 7. Kindred HU-500 harbors a recessive splice site mutation



Abbreviations

ACEI, angiotensin-converting enzyme inhibitors

CKD, chronic kidney disease

CsA, cyclosporine A

Csg, consanguineous

ESRD, end-stage renal disease

HD, hemodialysis

Hgb, hemoglobin

HUS, hemolytic-uremic syndrome

IVIG, intravenous immunoglobulin

MMF, mofetil mycophenolate

MPGN, membranoproliferative glomerulonephritis

n/a, not available

NC, non-consanguineous

ND, not done

NR, nephrotic-range

PD, peritoneal dialysis

PE, plasma exchange

PI, plasma infusion

PLT, platelets

sCr, serum creatinine

TMA, thrombotic microangiopathy

Tx, renal transplantation

URTI, upper respiratory tract infection

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