CASE REPORT

Five-year follow-up of a case of lipoprotein glomerulopathy with APOE Kyoto mutation

Ryosuke Usui¹ · Masaki Takahashi² · Kosaku Nitta³ · Minako Koike¹

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Abstract We report the case of a 34-year-old Japanese male with lipoprotein glomerulopathy (LPG). Renal biopsy showed LPG, and followed by a genetic analysis revealed a mutation in apolipoprotein E gene (APOE Kyoto; Arg25-Cys). We started treatment with probucol, bezafibrate, losartan, and allopurinol. Urinary protein decreased in response to treatment but has remained at about 1.27 ± 0.71 g/gCr, and a repeat biopsy which was performed 1 year after the first biopsy showed no clear evidence of pathological remission and complication of other glomerular disease. After 5 years of follow-up after the start of treatment, renal function has almost maintained without apparent deterioration. Interestingly, the course of the urinary protein level closely paralleled his triglyceride and cholesterol levels in a long-term. This observation suggests the importance of tight control of lipid profiles as a means of renoprotection in LPG patient.

Keywords Lipoprotein glomerulopathy · APOE Kyoto · Nephrotic syndrome

Introduction

Lipoprotein glomerulopathy (LPG) is an extremely rare hereditary glomerulopathy. A quarter of a century has passed since 1989, when Saito et al. proposed a new renal disease entity they called LPG [1], and about 150 cases have been reported thus far. Pathologically, the specific renal findings in LPG consist of lipid deposition as revealed by oil red-O staining and severe glomerular capillary dilatation. Many LPG patients have high serum apolipoprotein E (apoE) and triglyceride concentrations, and broad-beta hyperlipoproteinemia, so-called type III hyperlipidemia. The clinical features of the dyslipidemia of LPG patients, however, is different from those of typical dyslipidemia, it has been reported that hardly any LPG patients have severe atherosclerotic disorders as a complication. Although ApoE gene mutation is involved in the etiology and pathogenesis of LPG, it is not known how lipoprotein containing abnormal apoE gives rise to LPG. LPG has a poor renal prognosis. There is no established treatment for LPG, and although immunosuppressive and steroidal agents are ineffective, reports of successful treatment with anti-lipidemic medication have accumulated.

Case report

A 34-year-old Japanese male was referred to the Nephrology Division of the Yachiyo Medical Center of Tokyo Women's Medical University, in November 2010, because of urinary abnormalities and leg edema. Hematuria and proteinuria had been detected in annual medical checkups since 2007. In June 2010, the patient began to notice weight gain and lower limb edema, and he began to

✉ Ryosuke Usui
fzk11643@nifty.com

¹ Division of Nephrology, Department of Medicine, Yachiyo Medical Center, Tokyo Women's Medical University, 477-96 Yachiyo, Chiba, Japan

² Division of Nephrology, Minami-Senju Hospital, Tokyo, Japan

³ Department of Medicine IV, Tokyo Women's Medical University, Tokyo, Japan

experience general fatigue and malaise. These symptoms gradually worsened, and he was admitted to a local clinic in October 2010. Nephrotic syndrome was suspected because of a 4+ urinary protein by dipstick check and leg edema, and he was referred to our department for evaluation of renal disease. Initial laboratory findings (Table 1) showed nephrotic urinary abnormalities (urinary protein 5.1 g/gCr, 20–29 red blood cells/high-power field), hypoproteinemia (total protein 5.1 g/dL, serum albumin of 3.1 g/dL), mild renal dysfunction (serum creatinine 1.20 mg/dL), and dyslipidemia (total cholesterol 229 mg/dL, triglyceride (TG) 255 mg/dL, low-density lipoprotein (LDL) cholesterol 112 mg/dL, apoE 7.6 mg/dL), and then he was admitted for a thorough evaluation including renal biopsy and treatment. The family history revealed dyslipidemia in both parents, but no urinary abnormalities have been detected in other family members.

Physical examinations on admission revealed a blood pressure of 110/72 mmHg, slightly edematous lower limbs, height 164 cm and body weight 68.8 kg. No evidence of atherosclerotic complications, such as Achilles' tendon thickening, corneal rings, or xanthoma palpebrarum, were observed. We performed a renal biopsy and made a diagnosis of LPG based on the pathological findings such as marked glomerular capillary dilatation (Fig. 1a), positivity for oil-red O staining (Fig. 1b) and apoE in immunofluorescence (IF) (Fig. 1c), numerous oval-shaped lipid droplets in electron microscopy (EM) (Fig. 1d) in dilated glomerular capillaries. And we concluded that there was no evidence of concomitant thin basement membrane disease or chronic glomerulonephritis by irregular glomerular basement membrane thickness and no clear electron dense deposits in EM (Fig. 1d), negativity for IgA, IgG, and C3 in IF (Fig. 1e–g). A genetic analysis revealed the APOE Kyoto (Arg25Cys) mutation.

Table 1 Laboratory findings on admission

Urinalysis		Blood chemistry		Immunology	
specific gravity	1.005	HbA1c	5.1 (%)	IgG	480 (mg/dL)
pH	6.0	TP	5.1 (g/dL)	IgA	153 (mg/dL)
Protein	3+	Alb	3.1 (g/dL)	IgM	111 (mg/dL)
	5.1 (g/gCr)	AST	18 (IU/L)	CH50	39 (IU/mL)
Glucose	(–)	ALT	15 (IU/L)	C3	106 (mg/dL)
Occult blood	2+	LDH	188 (IU/L)	C4	36 (mg/dL)
Sediments		BUN	18.1 (mg/dL)	MPO-ANCA	<1.3 (IU/mL)
RBC	20–29/HPF	Cr	1.20 (mg/dL)	PR3-ANCA	<3.5 (IU/mL)
WBC	1–4/HPF	Na	141 (mEq/L)	ANA	<×40
Complete blood count		K	4.3 (mEq/L)	ds-DNA Ab.	<7 (IU/mL)
WBC	3560/μL	Ca	8.2 (mg/dL)	ss-DNA Ab.	<5 (AU/mL)
Neutrophils	54.2 (%)	iP	3.5 (mg/dL)	ASO	30 (IU/mL)
Lymphocytes	37.1 (%)	TC	229 (mg/dL)	Others	
Monocytes	3.9 (%)	TG	255 (mg/dL)	TSH	8.453 (μIU/mL)
Basophils	0.6 (%)	LDL	112 (mg/dL)	free T3	2.40 (pg/mL)
Eosinophils	4.2 (%)	apoE	7.6 (mg/dL)	BNP	23.5 (pg/mL)
RBC	438×10 ⁴ /μL	CRP	<0.03 (mg/dL)	Infection	
Hemoglobin	12.3 (g/dL)	eGFR	50.8 (mL/min/1.73m ²)	HBs antigen (–)	
Hematocrit	36.7 (%)	Coagulation		HCV antibody (–)	
Platelet	17.0×10 ⁴ /μL	PT (INR)	0.86	STS (–)	
Blood gas analysis		APTT	31.7 (s)	apoE genetic analysis	
pH	7.38	Fibrinogen	447 (mg/dL)	E3/E4 heterozygote	
HCO ₃ [–]	26.2 (mEq/L)	FDP	<3 (μg/mL)	APOE-Kyoto (Arg25Cys)	
B.E.	1.3 (mEq/L)				

WBC white blood cell, RBC red blood cell, BE bass excess, TP total protein, Alb albumin, AST aspartate aminotransferase, ALT alanine aminotransferase, LDH lactate dehydrogenase, BUN blood urea nitrogen, Cr creatinine, Na sodium, K potassium, Ca calcium, iP phosphate, TC total cholesterol, TG tryglyceride, LDL low density lipoprotein, apoE apolipoprotein E, CRP C-reactive protein, eGFR estimated glomerular filtration rate, PT(INR) thrombin time (international normalized ratio), APTT activated partial thromboplastin time, FDP fibrin and fibrinogen degradation products, IgG/A/M immunoglobulin G/A/M, CH50 complement hemolytic activity, C3/C4 complement, MPO-ANCA myeloperoxidase- anti-neutrophil cytoplasmic antibody, PR3 proteinase 3, ANA anti-nuclear antibody, ASO anti-streptolysin O, TSH thyroid stimulating hormone, BNP brain natriuretic peptide, HBs hepatitis B surface, HCV anti-hepatitis C, STS serological test for syphilis

Fig. 1 Renal biopsy findings: periodic acid-methenamine-silver stain (PAM) (a), oil-red O stain (b), apoE in IF (c), $\times 4000$ in EM (d), IgA (e), IgG (f), C3 (g) in IF, and at first biopsy specimens. And PAM stain at second biopsy (h) which was performed 1 year after the first biopsy

We started treatment with probucol 500 mg/day, bezafibrate 200 mg/day, losartan 25 mg/day, and allopurinol 50 mg/day. Urinary protein excretion decreased, but it has remained at about 1.26 ± 0.73 g/gCr throughout the subsequent clinical course. Since unusual microscopic hematuria as a finding of general LPG that did not respond to treatment was observed in our patient, we carried out second biopsy 1 year after the first biopsy, but we could not obtain new findings in a repeat renal biopsy. The second renal biopsy showed slight or no clear decrease in intraglomerular lipoprotein thrombi (Fig. 1h). No clear deterioration of renal function has been seen during the approximately 5-year follow-up period since the start of treatment (Fig. 2a).

Discussion

An ApoE gene mutation has been detected in most cases of LPG in which a genetic analysis has been performed. Ishigaki et al. [2] made mice lacking apoE gene developed LPG by adenoviral transduction of APOE Sendai. Removal of apoE containing lipoprotein by plasmapheresis, for example, by using a heparin-induced extracorporeal lipoprotein precipitation system [3] or protein A immunoabsorption [4], has been shown to decrease the urinary protein and improved the clinical condition of LPG patients. These reports [2–4] indicate that apoE plays a pivotal role in the etiology of LPG. However, despite being

an inherited disease, most cases of LPG are the adult-onset type, and no doubt healthy carriers exist [5, 6]. Furthermore, Ishimura et al. [7] found that not all mice into which had been APOE Sendai transduced developed LPG, suggesting that apoE mutation alone might not be sufficient to cause LPG and that second-hit factors such as another genetic disorder and/or environmental factors may be necessary. However, no second-hit factors have been found to date, and LPG may simply be an inherited disease with incomplete genetic penetrance. Moreover, a case of a relapse of nephrotic syndrome due to LPG 4 months after renal transplantation [8] and a case in which nephrotic syndrome due to LPG developed immediately after birth [9] have been reported. Specific histological changes of LPG were confirmed in only 6 days after transduction of APOE Sendai into APOE knockout mice in the experimental model [2], suggesting that LPG can develop very quickly in the presence or absence of some unknown triggers upon a background of apoE gene mutation. In any case, no triggers for the development of LPG are known.

Recently, Toyota et al. [5] reported a founder effect based on the results of a haplotype analysis of 13 LPG patients in 9 families with the APOE Sendai mutation. APOE Kyoto, which has been reported in various parts of the world, is the most common gene mutation in LPG. Interestingly, there are populations with the APOE Kyoto mutation in a very small area of China [6]. No haplotype analysis studies have yet been performed in this population but the possible involvement of a founder effect is

Fig. 2 The course of laboratory data. Serum creatinine (a); urinary protein and triglyceride (b); urinary protein and total cholesterol (c); and urinary protein and LDL cholesterol (d)

adequately concerned. The family history of our patient revealed dyslipidemia in both parents, but there is no clear evidence of the development of LPG in either parent. No genetic analyses of other family members have been performed because we could not obtain their informed consent. Although most patients with APOE Kyoto mutation are Chinese, none of our patients' relatives within the third degree of kinship are non-Japanese.

About 15 different apoE gene mutations have been reported in LPG patients thus far, and many of the mutations are in exon 4, which encodes the LDL receptor (LDLR) binding domain. ApoE proteins with a mutated 3-dimensional structure in the LDLR binding domain have less ability to bind the LDLR. Although there are no structural abnormalities in the LDLR binding domain of APOE Kyoto, its binding rate to the LDLR is only 10 % of the binding rate of recombinant apoE3 [10]. It is thought that the LDLR may not be directly involved in the pathogenesis of LPG because the role of the LDLR is in maintaining intracellular homeostasis [11]. On the other hand, we previously reported that macrophage migration is induced by ligand-independent activation of vascular endothelial growth factor receptor 1 by native LDL bound to the LDLR [12]. Since mutated apoE protein, which should be originally processed by internalization via the LDLR, causes LPG directly or indirectly, we cannot rule out the involvement of the LDLR in the etiology of LPG.

Involvement of the LDLR in the pathogenesis of LPG is still open to debate. In addition to the LDLR, other known receptors for apoE as their specific ligand include the apoE receptor, very-low-density lipoprotein receptor, and low-density lipoprotein-related protein (LRP). LRP contributes to macrophage migration [13]. Scavenger receptors play a role as clearance receptor for cholesterol with oxidative modification and accelerator for atherosclerosis. Lipoproteins and their receptors play important roles not only in lipid metabolism but in cell biology.

Recently, the role of macrophages in the pathogenesis of LPG has attracted our attention. Kanamaru et al. [14] demonstrated the graft-versus-host disease model in the Fc receptor gamma-deficient mice without apoE gene mutation developed LPG. Furthermore, Ito et al. [15] found that apoE and Fc receptor gamma double knockout mice exhibited LPG-like phenomena by adenoviral transduction of several apoE variants and Fc receptor gamma on macrophage might play an important role in the development of LPG.

Immunosuppressive agents including steroids, which are used as regular treatment for nephrotic syndrome or primary glomerulonephritis, are ineffective in LPG [16]. Although LPG is known to have a poor renal prognosis, case reports of successful treatment with lipid-lowering therapy have been increasing [17–23]. Interestingly, the course of the urinary protein level in our patient closely paralleled his TG and cholesterol levels (Fig. 2b–d),

suggesting the importance of tight control of TG and cholesterol levels as a means of renoprotection. There are several classes of drugs for the treatment of dyslipidemia such as statin, fibrate, probucol, niaspan, eicosapentaenoic acid and ezetimibe. Hu et al. [6] reported finding that fibrate was useful for maintaining renal function in LPG patients and it is likely that TG should be reduced lower than acceptable level as usual.

Drug selection itself may be more important in improving LPG patients' clinical condition, in addition to correcting the lipid profile. Fibrate is an agonist of peroxisome proliferator-activated receptor alpha (PPAR α), one of the nuclear receptor families. There have been several reports that PPAR α has a renoprotective effect [24–26]. The constitutive protein expression level of PPAR α in glomeruli is known to be extremely low, but its expression level is increased by a variety of stimuli, and it plays a role in renoprotection [27]. However, administration of fibrate to CKD patients tends to be avoided, because it is metabolized in the kidney and has adverse effects. There have been several reports of safe use of clonofibrate in continuous ambulatory peritoneal dialysis patients [28].

Our patient's serum lipid profile has elapsed at the acceptable level as usual (Fig. 2b–d), but proteinuria has persisted at about 1.27 ± 0.71 g/gCr, and no clear decrease in lipid deposits in the glomeruli has been observed in repeat biopsy specimens. LPG is known to be a renal disease with a poor prognosis, however, after 5 years of follow-up no clear renal function deterioration has been seen in our patient. These observations suggest that lipid-lowering treatment, including with fibrate, may improve the renal prognosis of LPG patients.

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Compliance with ethical standards

Informed consent Informed consent for DNA sequencing was obtained from the patient.

Conflict of interest The authors have declared that no Conflict of interest exists.

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