A case of apolipoprotein E Toyonaka and homozygous apolipoprotein E2/2 showing non-immune membranous nephropathy-like glomerular lesions with foamy changes

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Received: 17 October 2018 / Accepted: 4 January 2019 / Published online: 30 January 2019
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Abstract

A 47-year-old Japanese man with mild proteinuria was treated with an ACE inhibitor and antiplatelet agent for 7 years. However, urinary protein levels increased and renal biopsy was performed. Eight out of 20 glomeruli showed global or segmental sclerosis with foamy changes or bubbles, but with a different appearance to typical foam cells or lipoprotein thrombi. “Spike” formation, as observed in membranous nephropathy (MN), was segmentally detected in methenamine silver-stained sections. In an immunofluorescence study, weak linear patterns for IgG and scanty deposits for C3 were observed in glomeruli, but were not specific for immunogenetic MN. An electron microscopy study showed highly dense deposits in the subepithelial, subendothelial, and mesangial areas, in which microbubbles appeared under a higher magnification. Since this case exhibited hypertriglyceridemia and cholesterolemia with high serum apolipoprotein E (apoE) clinically and homozygous apoE2/2 by apoE phenotype and genotype analyses, apoE2 homozygote glomerulopathy was diagnosed and various lipid-lowering agents, e.g., probucol, fenofibrate, and ezetimibe, were administered. However, renal dysfunction gradually developed and peritoneal dialysis was initiated 11 years after the diagnosis. ApoE Toyonaka (Ser197Cys) and homozygous E2/2 were recently identified by direct DNA sequencing. Therefore, non-immune MN-like lesions may develop with the combination of these apoE mutations.

Keywords Apolipoprotein E Toyonaka · Homozygous apolipoprotein E2/2 · Apo E2 homozygote glomerulopathy · Lipoprotein glomerulopathy · Membranous nephropathy

Introduction

Apolipoprotein E (ApoE), a 34-kD glycoprotein, binds to cholesterol and triglycerides and constitutes lipoprotein structures. Accordingly, mutations in apoE have been implicated in various lipid abnormalities and induce atherosclerosis, leading to cardiovascular disorders and neurodegenerative disorders, e.g., Alzheimer’s disease [1, 2]. Lipoprotein glomerulopathy (LPG) [3] and apoE2 homozygote glomerulopathy [4] are kidney diseases caused by apoE mutants. Two cases of the combination of homozygous E2/2 and a novel apoE variant called apoE Toyonaka (Ser197Cys) have been reported to have another characteristic histology similar to membranous nephropathy (MN), but without obvious immune deposits [5, 6]. We herein present a case of the same combination of apoE mutants, in which various histological findings related to lipid abnormalities were recognized in addition to MN-like lesions.

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Case presentation

A 47-year-old Japanese man was diagnosed with mild proteinuria at an annual check-up and treated with an ACE inhibitor and antiplatelet agent for 7 years. Since urinary protein levels increased, he was admitted to Fukuiken Sais-eikai Hospital in 200× to undergo examinations, including renal biopsy. On admission, his height and weight were 177 cm and 62.5 kg, respectively. His blood pressure was 122/74 mmHg. No abnormal findings were identified in a physical examination. He had neither corneal opacities nor xanthoma. His laboratory findings were as follows: white blood cell count 5900/ μ L, (neutrophils 64.1%, lymphocytes 25.3%, eosinophils 3.4%), hemoglobin 14.7 g/dL, platelet count 235,000/ μ L, total protein 6.6 g/dL, albumin 3.9 g/dL, fasting blood glucose 100 mg/dL, serum creatinine 1.0 mg/dL, total cholesterol 252 mg/dL, triglycerides 417 mg/dL, and HDL-cholesterol 60 mg/dL. His serum apoE level was 7.5 mg/dL (normal range 2.4–5.2 mg/dL). Liver function and total complement were within normal ranges. Serum IgG, IgA, and IgM were 1013, 234, and 203 mg/dL, respectively. Anti-nuclear and anti-neutrophil cytoplasmic antibodies were negative. Urinary protein excretion was 2.4 g/day and urinary sediment RBC was 5–10/hpf. His parents, son, and daughter had not kidney diseases and did not agree to undergo an examination on apoE for the purpose of privacy protection.

Pathological findings

In renal biopsy, 20 glomeruli were detected in light microscopy specimens. Mild to moderate mesangial cell proliferation and mesangial matrix expansion were observed in most glomeruli. Eight glomeruli showed sclerotic changes, two of which were global, while the others were segmental with lipoprotein thrombi and bubbles (Fig. 1a). However, their appearance differed from foam cells or typical lipoprotein thrombi in LPG. “Spike” formation on the glomerular basement membrane (GBM), as observed in MN, was segmentally detected in methenamine silver-stained sections (Fig. 1b). In an immunofluorescence study, weak linear patterns for IgG and scanty deposits for C3 were detected in glomeruli, but were not specific for MN. An electron microscopy (EM) study showed highly dense deposits in the subepithelial, subendothelial, and mesangial areas, which had the appearance of microbubbles under a higher magnification (Fig. 2a, b). Lipoprotein thrombi with fine lucent granules were also observed by EM, but did not have the fingerprint-like layers characteristic of LPG (Fig. 2c, d).

Fig. 1 Light microscopic findings of the glomerulus (methenamine silver-stained sections). **a** Lipoprotein thrombi (arrowheads) and bubbling lesions (asterisks) are observed in the endothelium. **b** Spike formation was partly detected along the capillary walls (arrows)

Analysis of ApoE mutations and the clinical course

Since serum triglyceride, total cholesterol, and apoE levels were high, the apoE phenotype and genotype were initially examined. Plasma apoE phenotypes were analyzed by isoelectric-focusing polyacrylamide gel electrophoresis (IEF) and an immunoblotting analysis, as previously reported [7, 8]. The patient's sample showed the position of apoE2/2 (Fig. 3a). The apoE genotype was assessed by a restriction fragment length polymorphism (RFLP) analysis as described previously [7, 8]. PCR products were digested with the restriction enzyme *Hha*I. Genotype ϵ 2/2 was identified by 91- and 78-bp fragments (Fig. 3b).

Based on the findings of histology and the apoE analysis, the patient was diagnosed with apoE2 homozygote glomerulopathy [4] and was initially treated with probucol. However, improvements in dyslipidemia with a high

Fig. 2 Electron microscopic findings of the glomerulus. **a** Electron dense deposits are found in subepithelial (asterisks), subendothelial (arrowheads), and mesangial areas (arrows). **b** High magnification shows microbubbles in these dense deposits. **c** The capillary lumen

is occupied by a lipoprotein thrombus with foamy changes. **d** High magnification shows that the thrombus consists of fine lucent granules

apoE level or reductions in urinary protein levels were not observed. Fenofibrate and ezetimibe were administered, but did not ameliorate the patient's condition. Renal dysfunction gradually developed and peritoneal dialysis was initiated 11 years after the diagnosis.

Since similar cases of apoE Toyonaka (Ser197Cys) associated with homozygous E2/2 have recently been reported [5, 6], we re-analyzed apoE mutations with an RFLP analysis and direct DNA sequencing specific for apoE Toyonaka. ApoE Toyonaka (Ser197Cys) was confirmed by the RFLP analysis digested with *SacI* (Fig. 3c). Genomic DNA was amplified by a polymerase chain reaction (PCR) using the oligonucleotide primers, sense 5'-CGTGCGGGCCGCCAC TGTGAGCT-3' and antisense 5'-TCGCATGGCTGCAGG CTTCGGCGTTC-3'. The 335-bp fragment after cleavage by *SacI* suggested normal c.644C of apoE in codon 197 (lane 3). The 358- and 335-bp fragments after cleavage by *SacI* showed the heterozygous novel mutation of apoE Toyonaka (lane 2). The direct sequencing of apoE DNA was performed as described previously [9]. Based on the results obtained, we identified a homozygous polymorphism at codon 158

(ϵ 2/2: c.526 C>T: Arg158Cys) (data not shown) and heterozygous missense mutation (C to G) in exon 4 leading to the amino acid substitution Cys (TGC) for Ser (TCC) at codon 197 (c.644 C>G: p.S215C: Ser197Cys) (Fig. 3d). Therefore, apoE Toyonaka (Ser197Cys) associated with homozygous E2/2 was identified in this case.

Discussion

This is the third case report showing glomerular lesions with the combination of apoE Toyonaka and homozygous apoE2/2. The patient was born in a different area to the other two cases [5, 6] and had no consanguinity. Non-immune MN-like spike formation was common among these three cases. Subepithelial electron dense deposits between spikes were also similar to those in MN; however, microbubbles were observed under high magnification with EM instead of immune deposits by an immunofluorescence study. Although difficulties were associated with examining apoE in these deposits in the present case because renal biopsy

Fig. 3 Phenotype, genotype, and DNA sequences of apoE in the patient. **a** ApoE phenotype analysis. The patient was identified as E2/2 (lane 1). Controls (lanes 2, 3, and 4) show E2/3, E3/3, and E3/4, respectively. **b** ApoE genotype analysis by RFLP using *HhaI* as the restriction enzyme. The patient was identified as ε2/2 (lane 1). Controls (lanes 2, 3, and 4) show ε3/3, ε2/2, and ε3/4, respectively. Lane

M shows the marker. **c** RFLP for apoE Toyonaka using *SacI* as a restriction enzyme. Lanes M, 1, 2, and 3 show the marker, PCR only, the patient (apoE Toyonaka), and a normal control, respectively. **d** Sequence analysis of the *APOE* gene. A heterozygous missense mutation (c.644 C>G) in exon 4 leads to the amino acid substitution Cys (TGC, lower) for Ser (TCC, upper) at codon 197

was performed more than 10 years ago, tandem mass spectrometry and immunohistochemical staining in the previous case by Fukunaga et al. [5] revealed apoE in these deposits. Therefore, the glomerular dense deposits in the present case may also contain many apoE molecules. Since three cases of apoE Toyonaka and apoE2/2 showed similar non-immune MN-like findings, these histological changes may be specific to the combination of apoE Toyonaka and apoE 2/2.

The apoE molecule is composed of an N-terminal domain (NT) and C-terminal domain (CT). NT mainly contains an LDL receptor-binding region related to lipoprotein metabolism, and CT comprises the high-affinity lipid-binding region [2, 10]. These domains are joined by a protease-sensitive loop called a hinge region and maintained functionally and structurally [2, 10]. To date, LPG [3] and apoE2 homozygote glomerulopathy [4] characterized by lipoprotein thrombi and

foam cells, respectively, are regarded as representative renal glomerular diseases associated with apoE mutants in NT. On the other hand, apoE Toyonaka in the present case is a mutant of the hinge region, and appears to play an important role in the dissociation between NT and CT. Mutations in the hinge region have not been reported so far except for several non-invasive single-nucleotide polymorphisms (SNPs) [11] and further study should be needed for the dissociation between NT and CT. However, an altered hinge region may markedly affect the three-dimensional structure of apoE and cause disconnections not only between NT and LDL receptors, but also between CT and lipids [2, 10], because the structural and functional stability of apoE is mediated by the hinge region connecting NT and CT [12–14]. If the function of hinge region is suppressed by apoE Toyonaka, unstable apoE fragments without lipids are assumed to be present in

Table 1 Features of cases with apoE Toyonaka and homozygous E2

Patient	Sex	Age (yo)	UP (g/day)	HTG	Pathology		Therapy	Renal prognosis	Refs.
					EDD	FC/LT			
1.	Female	20	2.1	–	++	–/–	PSL+CyA	UP 0.5–2.5 after 9 years	[5]
2.	Male	79	5.4	+	+	++/+	PSL+CyA	Relapsed after 1 years	[6]
3.	Male	47	2.4	+	+	+/++	Lipid-lowering	ESRD after 11 years	Our case

UP urine protein, HTG hypertriglyceridemia, yo year-old, EDD electron dense deposits, FC/LT foam cells/lipoprotein thrombi, PSL prednisolone, CyA cyclosporin, ESRD end-stage renal disease, Ref. reference number

the blood and deposited as numerous electron dense granules in the mesangium and around GBM which include proteoglycan and other negatively charged substances. Particularly, since the apoE molecule is as small as 34 kDa and rich in arginine, one of the representative positively charged amino acids [15], it may cross the GBM, accumulate in the subepithelial area and form microbubble electron deposits. Therefore, non-immune MN-like lesions with spike formation may develop in these cases.

In comparisons between the present case and the two other cases, characteristics that differ from common MN-like lesions were noted (Table 1). The case reported by Fukunaga et al. [5] was normolipidemic and did not show foam cells or lipoprotein thrombi in the glomerulus. The case by Hirashima et al. [6] showed type III hyperlipoproteinemia and segmental glomerulosclerosis with numerous foam cells resembling apoE2 homozygote glomerulopathy. The present case was also similar to apoE2 homozygote glomerulopathy; however, lipoprotein thrombi were more prominent than in the case reported by Hirashima et al. [6]. Although apoE2 homozygote glomerulopathy is generally characterized by foam cell infiltration in the glomerulus, Sakatsume et al. [16] reported an atypical case of lipoprotein thrombus-like substances and electron dense deposits in the subendothelial and subepithelial areas. These findings resemble those of the present case and suggest the involvement of apoE2/2. Collectively, these findings indicate that the various glomerular changes observed in our case depend on the balance between two different mutations, apoE Toyonaka and homozygous E2/2. The accumulation of more cases is needed to clarify this mechanism.

In conclusion, we herein demonstrated that a case of apoE Toyonaka and homozygous apoE2/2 mutations had various glomerular lesions. Although histological findings slightly differed from those of the other two cases with the same mutant combination [5, 6], non-immune MN-like lesions were common among these three cases. Our case progressed to end-stage renal disease after a longer observation period than the other two cases, which suggests that the prognosis of this inherited condition is poor. On the other hand, it is reported that fibrates are effective for type III hyperlipoproteinemia in LPG and apoE2-homozygote glomerulopathy

[17]. If new cases are identified, such intensive treatment options available for this condition should be considered.

Funding None.

Compliance with ethical standards

Conflict of interest None of the authors have any competing interests to declare.

Human rights This article does not contain any studies with human participants performed by any of the authors.

Informed consent Written informed consent was obtained from the patient before the commencement of the phenotype and gene analyses of apoE.

References

- Dominiczak MH, Caslake MJ. Apolipoproteins: metabolic role and clinical biochemistry applications. *Ann Clin Biochem.* 2011;48:498–515.
- Tudorache IF, Trusca VG, Gafencu AV. Apolipoprotein E—a multifunctional protein with implications in various pathologies as a result of its structural features. *Comput Struct Biotechnol J.* 2017;15:359–65.
- Saito T, Matsunaga A, Oikawa S. Impact of lipoprotein glomerulopathy on the relationship between lipids and renal diseases. *Am J Kidney Dis.* 2006;47:199–211.
- Kawanishi K, Sawada A, Ochi A, Moriyama T, Mitobe M, Mochizuki T, Honda K, Oda H, Nishikawa T, Nitta K. Glomerulopathy with homozygous apolipoprotein E2: a report of three cases and review of the literature. *Case Rep Nephrol Urol.* 2013;3:128–35.
- Fukunaga M, Nagahama K, Aoki M, Shimizu A, Hara S, Matsunaga A, Muso E, Saito T. Membranous nephropathy-like apolipoprotein E deposition disease with apolipoprotein E Toyonaka (Ser197Cys) and a homozygous apolipoprotein E2/2. *Case Rep Nephrol Dial.* 2018;8:45–55.
- Hirashima H, Komiya T, Toriu N, Hara S, Matsunaga A, Saito T, Muso E. A case of nephrotic syndrome showing contemporary presence of apolipoprotein E2 homozygote glomerulopathy and membranous nephropathy-like findings modified by apoE Toyonaka. *Clin Nephrol Case Stud.* 2018;6:45–51.
- Oikawa S, Matsunaga A, Saito T, Sato H, Seki T, Hoshi K, Haya-saka K, Kotake H, Midorikawa H, Sekikawa A, Hara S, Abe K, Toyota T, Jingami H, Nakamura H, Sasaki J. Apolipoprotein E

- Sendai (arginine 145 → proline): a new variant associated with lipoprotein glomerulopathy. *J Am Soc Nephrol*. 1997;8:820–3.
8. Matsunaga A, Sasaki J, Komatsu T, Tsuji E, Moriyama K, Koga T, Arakawa K, Oikawa S, Saito T, Kita T, Doi T. A novel apolipoprotein E mutation, E2 (Arg25Cys), in lipoprotein glomerulopathy. *Kidney Int*. 1999;56:421–7.
 9. Kodera H, Mizutani Y, Sugiyama S, Miyata T, Ehara T, Matsunaga A, Saito T. A case of lipoprotein glomerulopathy with apoE Chicago and apoE (Glu3Lys) treated with fenofibrate. *Case Rep Nephrol Dial*. 2017;7:112–20.
 10. Narayanaswami V, Szeto SS, Ryan RO. Lipid association-induced N- and C-terminal domain reorganization in human apolipoprotein E3. *J Biol Chem*. 2001;276:37853–60.
 11. Masoodi TA, Al Shammari SA, Al-Muammar MN, Alhamdan AA. Screening and evaluation of deleterious SNPs in APOE gene of Alzheimer's disease. *Neurol Res Int*. 2012;2012:480609.
 12. Segall ML, Dhanasekaran P, Baldwin F, Anantharamaiah GM, Weisgraber KH, Phillips MC, Lund-Katz S. Influence of apoE domain structure and polymorphism on the kinetics of phospholipid vesicle solubilization. *J Lipid Res*. 2002;43:1688–700.
 13. Zhang Y, Vasudevan S, Sojitrawala R, Zhao W, Cui C, Xu C, Fan D, Newhouse Y, Balestra R, Jerome WG, Weisgraber K, Li Q, Wang J. A monomeric, biologically active, full-length human apolipoprotein E. *Biochemistry*. 2007;46:10722–32.
 14. Chen J, Li Q, Wang J. Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *Proc Natl Acad Sci USA*. 2011;108:14813–8.
 15. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science*. 1988;240:622–30.
 16. Sakatsume M, Kadomura M, Sakata I, Imai N, Kondo D, Osawa Y, Shimada H, Ueno M, Miida T, Nishi S, Arakawa M, Gejyo F. Novel glomerular lipoprotein deposits associated with apolipoprotein E2 homozygosity. *Kidney Int*. 2001;59:1911–8.
 17. Saito T, Matsunaga A, Ito K, Nakashima H. Topics in lipoprotein glomerulopathy: an overview. *Clin Exp Nephrol*. 2014;18:214–7.

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