Membranous Nephropathy-Like Apolipoprotein E Deposition Disease with Apolipoprotein E Toyonaka and Homozygous Apolipoprotein E2/2 without Dyslipidemia, with Characteristic Electron-Dense Deposits

Akihiko Koshino¹, ², Chikako Takaeda ³, Takahiro Matsuno¹, ², Shinji Kitajima ³, Yasunori Iwata ³, Norihiko Sakai ³, Kiyotaka Nagahama ⁴, Yo Niida ⁵, Takao Saito ⁵, Hitoshi Yokoyama ⁶, Takashi Wada ²

¹Department of Nephrology and Hypertension, Public Central Hospital of Matto Ishikawa, Hakusan, Japan; ²Department of Nephrology and Laboratory Medicine, Kanazawa University, Kanazawa, Japan; ³Departments of Pathology, Kyorin University School of Medicine, Tokyo, Japan; ⁴Center for Clinical Genomics, Kanazawa Medical University Hospital, Uchinada, Japan; ⁵Sanko Clinic, Fukuoka, Japan; ⁶Department of Nephrology, Kanazawa Medical University School of Medicine, Uchinada, Japan

Keywords
Apolipoprotein E Toyonaka · Homozygous apolipoprotein E2/2 · Ser197Cys · Lipid · Glomerular basement membrane

Abstract
Recently, several cases of novel apolipoprotein E (apoE)-related glomerular disease known as membranous nephropathy (MN)-like apoE deposition disease with apoE Toyonaka (Ser197Cys) and homozygous apoE2/2 have been reported. However, the clinical and pathological characteristics are uncertain due to the small number of reports. Here, we report an additional case with various clinical and pathological characteristics. A 28-year-old Japanese man with mild proteinuria and hematuria underwent a kidney biopsy. Examination under a light microscope revealed mesangial proliferation, mesangial matrix expansion, and segmental spike lesion. An immunofluorescence study showed no immunoglobulin or complement depositions. In the electron microscopic (EM) examination, massive deposits with various electron densities in the subepithelial, subendothelial, and paramesangial areas were more prominent than
those reported in previous cases, which resembled microbubbles or microcysts on higher magnification. The glomerular basement membrane (GBM) structure was partly degenerated by these deposits. Serum triglyceride and cholesterol levels were within the normal range. However, the serum apoE concentration was significantly high, and glomerular apoE accumulation was detected in immunohistochemistry. The DNA sequence revealed apoE Toyonaka and homozygous apoE2/2 similar to that of the previous cases with MN-like apoE deposition disease. MN-like apoE deposition disease can manifest as only mild hematuria and proteinuria without dyslipidemia. Various characteristic deposits associated with GBM degeneration can be observed in the EM study.

Introduction

Lipid-related glomerular disorders are frequently associated with apolipoprotein E (apoE), one of the lipoprotein components. ApoE2 homozygote glomerulopathy shows marked foam cell infiltration and is found in individuals expressing apoE2/E2, which occasionally induces type III hyperlipoproteinemia [1]. Lipoprotein glomerulopathy is characterized by lamellated lipoprotein thrombi without foam cells and is primarily associated with heterozygous apoE variants, such as apoE Sendai (Arg145Pro) and apoE Kyoto (Arg25Cys) [2, 3].

Recently, several cases of apoE Toyonaka (Ser197Cys) and homozygous apoE2/2 have been reported [4–6]. These cases demonstrated spike formation in periodic acid-methenamine silver-stained sections and electron-dense deposits (EDDs) in subepithelial and subendothelial areas in common. The EDDs resembled microbubbles or microcysts on higher magnification. Due to the absence of a specific immunoglobulin and complement depositions in the immunofluorescence (IF) study and the identification of glomerular apoE accumulation in immunohistochemical (IHC) studies and tandem mass spectrometry, these cases were categorized as apoE-related disease and termed as membranous nephropathy (MN)-like apoE deposition disease [7]. However, there is no clear information regarding the clinical and pathological presentations due to the small number of reports.

Here, we report an additional case of the same combination of apoE mutants, where chance proteinuria and hematuria without dyslipidemia were found. Electron microscopic (EM) study showed that massive deposits with various electron densities in the subepithelial, subendothelial, and paramesangial areas were more prominent than those observed in previous cases [4–6], which resembled microbubbles or microcysts on higher magnification. The glomerular basement membrane (GBM) structure was partly degenerated by these deposits.

Case Report/Case Presentation

The patient was a 28-year-old Japanese man without significant medical history and family history, including low birth weight, kidney disease, and dyslipidemia. For the past 4 years, his annual health surveillance indicated proteinuria and hematuria. He was admitted to the Public Central Hospital of Matto Ishikawa for examination, including a renal biopsy. At admission, his height, weight, and body mass index were 171 cm, 72.1 kg, and 24.7 kg/m2, respectively. His blood pressure and pulse rate were 122/69 mm Hg and 75/min, respectively.
There were no abnormal findings on his physical examination, including purpura, corneal opacity, and xanthoma. The laboratory findings on admission are described in Table 1. His proteinuria and hematuria were relatively mild, and the levels of serum creatinine, triglyceride, and cholesterol were within the normal range. Cryoglobulin was positive, although the type of cryoglobulin was not assessed and complement levels were not low.

Four out of 33 glomeruli showed global sclerosis in the light microscopic study. Neither endocapillary nor extracapillary hypercellularity was marked. Most glomeruli showed moderate mesangial cell proliferation and mesangial matrix expansion (shown in Fig. 1a). Spike formation and bubbling on the GBM were focally detected in periodic acid-methenamine silver-stained sections (shown in Fig. 1b). Neither foam cells nor lipoprotein thrombi were

| Table 1. Laboratory findings on admission (fasting condition) |
|-------------------------------------------------------------|
| **Urinalysis** | **Blood Chemistry (2)** |
| pH | 6.0 | AST, U/L | 18 (13–33) |
| Protein | (1+) | ALT, U/L | 23 (8–42) |
| 0.8 g/day | LDH, U/L | 147 (119–229) |
| Occult blood | (1+) | γ-GTP, U/L | 29 (10–47) |
| Casts | Glass | Total cholesterol, mg/dL | 157 (128–419) |
| | Epithelial | Triglyceride, mg/dL | 141 (30–149) |
| RBC, HPF | 1–4 | HDL cholesterol, mg/dL | 52 (40–99) |
| WBC, HPF | <1 | CRP, mg/dL | 0.15 (<0.30) |
| Urine Bence-Jones protein | (-) | Glucose, mg/dL | 96 (60–110) |

**Casual blood count**

| WBC, /µL | 7,010 (3,800–8,800) | HBs antigen | (-) |
| RBC, ×10⁴/µL | 493 (440–560) | Anti-HCV antibody | (-) |
| Hb, g/dL | 15.3 (14.0–18.0) | Serological tests |
| Ht, % | 45.4 (40.0–48.0) | IgG, mg/dL | 919 (870–1,700) |
| Plt, ×10⁴/µL | 38.1 (11.0–36.0) | IgA, mg/dL | 267 (110–410) |

**Blood chemistry (1)**

| Na, mEq/L | 142 (135–149) | Anti-nuclear antigen | <×20 (<×40) |
| K, mEq/L | 4.6 (3.5–4.9) | C3, mg/dL | 122 (65–135) |
| Cl, mEq/L | 103 (96–108) | C4, mg/dL | 30 (13–35) |
| Total protein, g/dL | 7.0 (6.7–8.3) | CH50, CH50U/mL | 52.8 (30.0–46.0) |
| Albumin, g/dL | 4.3 (4.0–5.0) | Anti-ds-DNA antibody, IU/mL | <10 (<12) |
| BUN, mg/dL | 11 (8–22) | MPO-ANCA, U/mL | <1.0 (<3.5) |
| Creatinine, mg/dL | 0.79 (0.6–1.0) | PR3-ANCA, U/mL | <1.0 (<2.0) |
| Estimated GFR, mL/min/1.73 m² | 96.5 | Cryoglobulin | (+) |
| Uric acid, mg/dL | 4.3 (3.6–7.0) | Serum IEP | (-) |

RVs are given in parentheses.

RBC, red blood cell; WBC, white blood cell; HPF, high power field; Hb, hemoglobin; Ht, hematocrit; Plt, platelets; BUN, blood urea nitrogen; GFR, glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase; HDL, high-density lipoprotein; CRP, C-reactive protein; HCV, hepatitis C virus; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; MPO-ANCA, myeloperoxidase antineutrophil cytoplasmic antibodies; PR3-ANCA, proteinase 3 antineutrophil cytoplasmic antibodies; IEP, immune electrophoresis.
marked. In the IF study, immunoglobulins and complement components were not recognized (data not shown). The EM study revealed massive deposits with various electron densities in the subepithelial, subendothelial, and paramesangial areas, accompanied by foot process effacement (shown in Fig. 2a–d). On higher magnification, the EDDs in the subepithelial area resembled microbubbles or microcysts, and there were no short fibrillary substructures suggestive of cryoglobulin nephritis. The structure of the GBM was degenerated with these deposits in some parts of the glomerular capillary wall (shown in Fig. 2b, c).

Despite normal serum triglyceride and cholesterol levels, the additional lipid analysis revealed high serum apoE concentration (apoA1 157 mg/dL [reference value [RV]: 126–165 mg/dL], apoA2 30.8 mg/dL [RV: 25.9–35.7 mg/dL], apoB 68 mg/dL [RV: 73–109 mg/dL], apoC2 5.7 mg/dL [RV: 1.8–4.6 mg/dL], apoC3 8.6 mg/dL [RV: 5.8–10.0], and apoE 8.0 mg/dL [RV: 2.7–4.3 mg/dL]). As these clinical and pathological findings were similar to those of the previous MN-like apoE deposition disease cases with apoE Toyonaka and homozygous apoE2/2 [4–6], an IHC study and DNA sequencing of apoE were performed. Widespread apoE accumulation in both the GBM and mesangial area was detected in the IHC.
study using an anti-apoE antibody (Novus Biologicals, CO, USA) (shown in Fig. 1c). In the analysis of the APOE gene, the whole genome region of \textit{APOE} was amplified by long-range polymerase chain reaction from the peripheral blood DNA. DNA sequences were screened by next-generation sequencing and validated by Sanger sequencing. Both codons 112 and 158 showed the amino acid cysteine (Cys) (shown in Fig. 3), which indicated that the isoform of apoE was apoE2/E2 [8, 9]. A heterozygous missense mutation (c.644C>G) that results in an amino acid substitution known as apoE Toyonaka (Ser197Cys) was detected (shown in Fig. 3).

The patient was prescribed diet therapy, including calorie and sodium restriction, as a treatment for chronic kidney disease during admission. He was finally diagnosed with MN-like apoE deposition disease based on the histological findings and APOE analysis. Unfortunately, the patient requested to withhold treatment and follow-up after the final diagnosis and stopped visiting the hospital.

\textbf{Discussion/Conclusion}

To the best of our knowledge, this case is the fourth report of MN-like apoE deposition disease with apoE Toyonaka and homozygous apoE2/2 in the literature. We observed two important issues. Clinically, this disease can manifest as only mild hematuria and proteinuria without dyslipidemia. Histologically, in the EM study, there were various characteristic features of subepithelial, subendothelial, and paramesangial deposits associated with GBM degeneration in addition to the common features of previous cases.
This disease can present as only mild hematuria and proteinuria without dyslipidemia. The three previously reported cases showed a relatively high amount of proteinuria of ≥2.0 g/day at the time of diagnosis, although it was 0.8 g/day in the present case (Table 2). The previous cases showed increases in proteinuria and decreases in renal function in their long-term course. In the case reported by Fukunaga et al. [4], the second biopsy revealed a more robust apoE deposition than the first biopsy performed 9 years earlier. These findings might imply that our case was in an early phase of the disease. Regarding dyslipidemia, which is commonly seen in other apoE-related diseases, one of the previous cases showed no dyslipidemia and the other two cases showed both hypertriglyceridemia and hypercholesterolemia (Table 2). Due to the structural change in apoE caused by apoE Toyonaka, this disease may not be accompanied by dyslipidemia. The apoE molecule comprises an N-terminal domain (NT; amino acids 1–191), a hinge region (amino acids 192–215), and a C-terminal domain (CT; amino acids 216–299). NT includes an LDL receptor and heparan sulfate proteoglycan-binding region, and CT is critical for lipid binding [10]. The hinge region plays an indispensable role in the binding of both domains [10]. Due to their inability to bind to the LDL receptor, ApoE2 (Arg158Cys) and major LPG mutants (apoE Kyoto [Arg25Cys] and Sendai [Arg145Pro]),

![Fig. 3. Sequence analysis of the APOE gene. A heterozygous missense mutation (NM 000041.4:c.644C>G) which led to an amino acid substitution known as apoE Toyonaka (Ser197Cys) was detected. Cysteine at both codon 112 and 158 implied that the isoform was apoE2/E2.](image-url)

**Table 2.** Features of cases with MN-like apoE deposition disease with apoE Toyonaka and homozygous E2

| Patient | Sex | Age, yo | UP, g/day | HU, HPF | HTG | HCL | apoE, mg/dL | Refs |
|---------|-----|---------|-----------|---------|-----|-----|-------------|------|
| 1       | F   | 20      | 2.1       | 50–99   | -   | -   | 10.4        | [4]  |
| 2       | M   | 79      | 5.4       | 10–19   | +   | +   | 13.6        | [5]  |
| 3       | M   | 47      | 2.4       | 5–9     | +   | +   | 7.5         | [6]  |
| 4       | M   | 28      | 0.8       | 5–9     | -   | -   | 8.0         | our case |

yo, years old; UP, urine protein; HU, hematuria; HPF, high power field; HTG, hypertriglyceridemia; HCL, hypercholesterolemia; apoE, apolipoprotein E; Refs, reference number; F, female; M, male.
which are located in the NT, induce dyslipidemia [7, 11]. However, apoE Toyonaka (Ser197Cys) is a mutation in the hinge region. The deformed hinge region may markedly affect the three-dimensional structure of apoE and cause dysfunction of both NT and CT [7]. This disconnection of CT and lipid may negate the type III hyperlipidemia due to apoE2 homozygote [7]. In fact, no lipid abnormalities were observed in one of the previous cases with apoE Toyonaka and homozygous apoE2/2 [4].

Suspecting the MN-like apoE deposition disease clinically, especially in an early phase of the disease, may be difficult due to the lack of specific laboratory tests and the low awareness of this disease. In all reported cases, including our case, the serum apoE concentration was high, regardless of the presence of dyslipidemia (Table 2). Meanwhile, the serum concentration of apoE can be markedly elevated in nephrotic syndrome [12]. Altogether, measurement of serum apoE concentration might be a clue to suspect the MN-like apoE deposition disease in nonnephrotic conditions.

In the EM study, we found further findings compared with the previous cases of the MN-like apoE deposition disease. In the previous cases, high electron deposits were mainly seen in the subepithelial area. Our case showed massive electron deposits with various densities, consisting of microbubbles and microcysts in the subepithelial, subendothelial, and paramesangial areas, inducing GBM degeneration. A wide variety of these findings were reported in one case with homozygous apoE2/2 [13]. However, the exact reason for the occurrence of such features in cases with apoE abnormalities is unclear. Our case did not show foam cell or lipoprotein thrombi that were observed in the two previous cases [5, 6]. Animal experiments revealed the importance of podocyte injury in foam cell formation based on hypercholesterolemia [14]. Apart from normal cholesterol levels, the absence of medical history associated with podocyte injury, such as hypertension, obesity, and low birth weight, could explain this difference. Clinically, our case appears to have been examined in the early phase of the MN-like apoE deposition disease because of mild proteinuria of < 1.0 g/day. There is a discrepancy between mild clinical features and marked histological findings. In the future, analyses of other cases or animal models of this disease may address these issues.

This case presented with cryoglobulinemia without any clinical or hematological evidence of vasculitis. The negative IF study and absence of a fibrillary structure in the EM study suggest that the pathological changes in the kidney in the light microscopic study are irrelevant to cryoglobulinemia. Cryoglobulin can be detected in the serum of healthy individuals with normal levels of complement; however, the symptoms of cryoglobulin vasculitis during follow-up could emerge [15].

A limitation of this case report is that it does not include information regarding the clinical course after diagnosis as the patient stopped visiting the hospital. The previous two cases showed a gradual decrease in kidney function, despite receiving either immunosuppressive therapy or lipid-lowering drugs [4, 6]. One case was started on dialysis 11 years after diagnosis [6]. The other case was treated with prednisolone, cyclosporine, and fibrate, which led to a decrease in proteinuria [5]. These data were explained to the patient, and he was informed that he could start treatment at any time if he changed his opinion.

In conclusion, we report an additional case of MN-like apoE deposition disease with apoE Toyonaka and homozygous apoE2/2. Clinically, this case showed only mild proteinuria and hematuria without dyslipidemia, but there was a wide variety of histological findings as well as MN-like features characterized by subepithelial high EDDs. To date, all the four cases of MN-like apoE deposition disease have been reported from the west-central part of the mainland of Japan. Considering that the prevalence of the MN-like apoE deposition disease in this area might be high, there is a need for further collection and investigation of similar cases.
Acknowledgments

The authors want to thank Enago (www.enago.jp) for the English language review.

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Ethical approval is not required for this study in accordance with the local guideline of the Public Central Hospital of Matto Ishikawa.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The authors received no specific funding for this work.

Author Contributions

Akihiko Koshino, Chikako Takaeda, and Takahiro Matsuno analyzed and interpreted the patient clinical data. Akihiko Koshino and Chikako Takaeda wrote the first draft of the manuscript. Shinji Kitajima, Yasunori Iwata, Norihiko Sakai, Kiyotaka Nagahama, Takao Saito, Hitoshi Yokoyama, and Takashi Wada performed the histological examination and contributed to pathological interpretation. Yo Niida performed APOE gene analysis.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1. Kawanishi K, Sawada A, Ochi A, Moriyama T, Mitobe M, Mochizuki T, et al. Glomerulopathy with homozygous apolipoprotein E2: a report of three cases and review of the literature. Case Rep Nephrol Urol. 2013;3(2):129–35.
2. Oikawa S, Matsunaga A, Saito T, Sato H, Seki T, Hoshi K, et al. Apolipoprotein E Sendai (arginine 145-->proline): a new variant associated with lipoprotein glomerulopathy. J Am Soc Nephrol. 1997;8(5):820–3.
3. Matsunaga A, Sasaki J, Komatsu T, Kanatsu K, Tsuji E, Moriyama K, et al. A novel apolipoprotein E mutation, E2 (Arg25Cys), in lipoprotein glomerulopathy. Kidney Int. 1999;56(2):421–7.
4. Fukunaga M, Nagahama K, Aoki M, Shimizu A, Hara S, Matsunaga A, et al. Membranous nephropathy-like apolipoprotein E deposition disease with apolipoprotein E Toyonaka (Ser197Cys) and a homozygous apolipoprotein E2/2. Case Rep Nephrol Dial. 2018;8(1):45–55.
5. Hirashima H, Komiya T, Toriu N, Hara S, Matsunaga A, Saito T, et al. A case of nephrotic syndrome showing contemporary presence of apolipoprotein E2 homozygote glomerulopathy and membranous nephropathy-like findings modified by apolipoprotein E Toyonaka. Clin Nephrol Case Stud. 2018;6:45–51.
6 Kato T, Ushiogi Y, Yokoyama H, Hara S, Matsunaga A, Muso E, et al. A case of apolipoprotein E Toyonaka and homozygous apolipoprotein E2/2 showing non-immune membranous nephropathy-like glomerular lesions with foamy changes. CEN Case Rep. 2019;8(2):106–11.

7 Saito T, Matsunaga A, Fukunaga M, Nagahama K, Hara S, Muso E. Apolipoprotein E-related glomerular disorders. Kidney Int. 2020;97(2):279–88.

8 Weisgraber KH, Rall SC, Mahley RW. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem. 1981;256(17):9077–83.

9 Rall SC, Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. J Biol Chem. 1982;257(8):4171–8.

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(41):37853–60.

11 Mahley RW, Huang Y, Rall SC. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. J Lipid Res. 1999;40(11):1933–49.

12 Bruschi M, Catarsi P, Candiano G, Rastaldi MP, Musante L, Scolari F, et al. Apolipoprotein E in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. Kidney Int. 2003;63(2):686–95.

13 Sakatsume M, Kadomura M, Sakata I, Imai N, Kondo D, Osawa Y, et al. Novel glomerular lipoprotein deposits associated with apolipoprotein E2 homozygosity. Kidney Int. 2001;59(5):1911–8.

14 Hara S, Kobayashi N, Sakamoto K, Ueno T, Manabe S, Takashima Y, et al. Podocyte injury–driven lipid peroxidation accelerates the infiltration of glomerular foam cells in focal segmental glomerulosclerosis. Am J Pathol. 2015;185(8):2118–31.

15 Muchtar E, Magen H, Gertz MA. How I treat cryoglobulinemia. Blood. 2017;129(3):289–98.