Focal segmental glomerulosclerosis with heterozygous apolipoprotein E5 (Glu3Lys)

Masaru Sasaki¹ · Tetsuhiko Yasuno¹ · Kenji Ito¹ · Akira Matsunaga² · Satoshi Hisano³ · Yasuhiro Abe¹ · Katsuhisa Miyake¹ · Kosuke Masutani¹ · Hitoshi Nakashima¹ · Takao Saito⁴

Received: 7 March 2018 / Accepted: 16 April 2018 / Published online: 8 May 2018
© Japanese Society of Nephrology 2018

Abstract

Apolipoprotein (apo) E5 is a rare apoE isoform. The apoE5 (Glu3Lys) variant, which is caused by the substitution of lysine with glutamic acid at codon 3, has a relative frequency of 0.1% in Japan. Previous studies have reported that apoE5 (Glu3Lys) is associated with hyperlipidemia and cardiovascular diseases, but this isoform has higher LDL receptor-binding activity than that of normal apoE3. Nephropathy associated with apoE5 (Glu3Lys) alone has not yet been reported. We present a case of a 51-year-old man with nephrotic syndrome. On renal biopsy, three glomeruli showed segmental sclerosis with hypertrophy of podocytes and intracapillary marked infiltration of intraglomerular foam cells. These findings were compatible with focal segmental glomerulosclerosis (FSGS). The patient had mild diabetes mellitus and monoclonal gammopathy of undetermined significance, but there were no specific findings of nephropathy related to these diseases. Various factors are involved in the pathogenesis of FSGS, including dyslipidemia and apoE activity. Our findings suggest that abnormal lipid metabolism by ApoE5 (Glu3Lys) is involved in the onset of FSGS.

Keywords ApoE isoform · Foam cells · Hyperlipidemia · Podocyte injury · Variant

Introduction

Human apolipoprotein (apo) E is a glycoprotein of 34 kD containing 299 amino acids; it forms a lipoprotein with tri-glycerides and cholesterol and plays a central role in lipoprotein metabolism. ApoE has three major isoforms, i.e., apoE2, E3, and E4, and three minor isoforms (E1, E5, and E7) have also been reported. In Japan, an apoE5 variant caused by the substitution of lysine to glutamic acid at codon 3 has been observed, with a relative frequency of approximately 0.1% [1].

Previous studies have shown that apoE5 (Glu3Lys) is involved in hyperlipoproteinemia and cardiovascular diseases, although this isoform has higher LDL receptor-binding activity than that of normal apoE3 [2, 3]. Hyperlipidemic kidney disease is related to apoE5, and several cases of lipoprotein glomerulopathy (LPG) have recently been reported. However, other mutations specific to LPG, such as apoE-Chicago [4] and apoE-Sendai [5], have been detected in these cases and therefore the causative role of apoE5 is not clear.

We report a case of hyperlipidemic focal segmental glomerulosclerosis (FSGS) associated with heterozygous apoE5 (Glu3Lys) and wild-type apoE3. A variety of factors are implicated in the pathogenesis of FSGS [6], including roles of dyslipidemia [7] and apoE [8], and abnormal lipid metabolism by ApoE5 (Glu3Lys) may be involved in the onset of FSGS, as observed in this case.

✉ Tetsuhiko Yasuno
yasuno9584@fukuoka-u.ac.jp

¹ Division of Nephrology and Rheumatology, Department of Internal Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

² Department of Laboratory Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

³ Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

⁴ Sanko Clinic, Fukuoka, Japan

Case report

A 51-year-old Japanese man was admitted to Fukuoka University Hospital for the examination of kidney disease. He had been treated for hypertension, diabetes, dyslipidemia, and hypothyroidism for 15 years by a local practitioner, and nephrotic syndrome was suspected based on hypoproteinemia and leg edema 10 months before transfer. Although he had a past history of diabetes mellitus since approximately 10 years, he took an oral hypoglycemic agent, and findings of diabetic retinopathy and neuropathy were lacking. In addition, there was no family history of diabetes, dyslipidemia, or kidney diseases.

On admission, the patient exhibited a height of 174 cm, weight of 78.9 kg, and blood pressure of 184/105 mmHg. Physical examination revealed no particular abnormalities, other than pitting edema of the lower extremities. Systemic manifestations characteristic of lipidosis, such as corneal arcus and xanthoma, were not recognized. Laboratory findings were as follows: white blood cell count, 6,600/ μ L (neutrophils, 76.7%; lymphocytes, 18.6%; eosinophils, 0.3%); hemoglobin, 14.0 g/dL; platelet count, 227,000/ μ L; total protein, 5.3 g/dL; albumin, 2.5 g/dL; fasting blood glucose 102 mg/dL; hemoglobin A1c, 6.0%; creatinine, 1.06 mg/dL; urea nitrogen, 18 mg/dL; triglycerides, 141 mg/dL; low-density lipoprotein cholesterol, 208 mg/dL; high-density lipoprotein cholesterol, 81 mg/dL. Liver function was within normal ranges. Serum immunoglobulin and complement levels were as follows: IgG, 701 mg/dL; IgA, 182 mg/dL; IgM, 44 mg/dL; C3, 160 mg/dL; C4, 47 mg/dL. Results for anti-nuclear antibodies were

negative. The serum apoE level was 5.7 mg/dL (normal range 2.7–4.3 mg/dL). A lipoprotein analysis by agarose gel electrophoresis showed a pattern consistent with type IIa hyperlipoproteinemia, with a slight elevation of the pre β band. Urinalysis showed substantial protein excretion (12.4 g/gCr) and mild hematuria in sediments (red blood cells 5–9/hpf).

An M-spike was detected by serum protein electrophoresis, and serum immunoelectrophoresis showed an M-bow of IgG- κ type. Bone marrow aspiration and biopsy revealed 3% plasma cells with normal cytogenesis. The skeletal survey was negative for lytic lesions or pathological fractures. Based on these findings, the patient was diagnosed with monoclonal gammopathy of undetermined significance (MGUS).

Pathological findings

The patient underwent percutaneous kidney biopsy 2 days after admission. Light microscopic observation revealed that the specimen contained 31 glomeruli, 10 of which showed global sclerosis. Three glomeruli showed segmental sclerosis with hyperplasia of podocytes and intracapillary marked infiltration of intraglomerular foam cells. These findings were compatible with FSGS, cellular variant (Fig. 1a–c). Characteristic findings of diabetic glomerulosclerosis or monoclonal gammopathy were not observed, although mild-to-moderate interstitial fibrosis with tubular atrophy, and arteriosclerosis were observed. Based on electron microscopy, foam cells containing bubbled lipid droplets were observed in the glomeruli, but there were no electron-dense deposits (Fig. 1c). In addition, the lipoprotein thrombus

Fig. 1 Histological findings of the renal biopsy. **a–c** Light microscopy. **a** Foam cells (arrows) are observed in a segmental sclerotic area of right glomerulus (Masson-trichrome, $\times 200$). In the same glomerulus, segmental sclerosis (**b**, asterisk) and hyperplasia of podocytes (**c**, arrowheads) are more definite in other sections **b**, periodic acid Schiff; **c**, periodic acid methenamine silver. **d** Immunohistochemical study. CD68⁺ macrophages (arrows) are consistent with foam cells in the serial section of (**a**). **e** Electron microscopy. Foamy changes are also seen in a sclerotic area of the glomerulus

specific to LPG was not detected in any glomeruli. Neither immunoglobulins and light chains nor complements were identified by immunofluorescence.

Phenotypic, genotypic and DNA sequencing analyses of apoE

Since the serum apoE level exceeded the normal range, apoE phenotypes were analyzed by isoelectric focusing polyacrylamide gel electrophoresis, as previously described [4, 5]. Different from apoE3/3 (a wild-type control), the apoE3/5 phenotype was detected in the patient (Fig. 2a).

We obtained written informed consent for a genomic analysis. The apoE genotype was determined by a restriction fragment length polymorphism (RFLP) analysis, as described previously [4]. Genomic DNA was amplified by polymerase chain reaction (PCR) using oligonucleotide primers (sense 5'-ACGCGGGCACGGCTGTCCAA-GGA-3' and antisense 5'-TCGCGGGCCCCGGCCTGGTACAC-3'). The PCR products were digested with the restriction enzyme *HhaI*. Genotype ϵ 3/3 in the patient was identified by 91- and 48-bp fragments (Fig. 2b), and other major isoforms, apoE2 (Arg158Cys) and apoE4 (Cys112Arg), were excluded.

Sequencing of *APOE* DNA was performed as follows. Three fragments of genomic DNA containing the coding sequence of mature *APOE* were amplified by PCR using

the following primers for *APOE*: 5'-GCTTTCCAAGTG ATTAACCGACT-3' and 5'-AGAGCTAAAGCCAGG AGTCAG-3' for exon 3, and 5'-CCTCTTGGGTCTCTC TGGCT-3', 5'-CTGCTCCTTCACCTCGTCCA-3', 5'-GCA GTACCGCGGCGAGGTGCAGG-3', and 5'-GATCGT GCCACTGCACTCTA-3' for exon 4. Amplified DNA fragments were purified using a PCR Purification Kit (Qiagen, Hilden, Germany) and directly sequenced using a Genetic Analyzer 3130xl (Thermo Fisher Scientific, Waltham, MA, USA) with a BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific). A substitution of lysine with glutamic acid at codon 3 of *APOE* corresponded to the point mutation of heterozygous apoE5 (Glu3Lys), a rare apoE isoform (Fig. 2c) Unfortunately, since his parents had died and their apo E could not be analyzed, it was unclear whether apoE5 mutation was de novo or hereditary.

Clinical course

Based on the kidney biopsy findings, phenotype, and DNA sequence analyses of *APOE*, we diagnosed the patient to have FSGS with heterozygous apoE5/3. The patient continued taking an angiotensin receptor blocker and HMG-CoA reductase inhibitor. Renal function was stable with nephrotic syndrome for 1 year.

Fig. 2 **a** ApoE phenotype determined by isoelectric focusing polyacrylamide gel electrophoresis (IEF). Lane 1, apoE2/2 (patient); Lane 2, apoE3/3 (wild type); Lane 3, apoE4/4; Lane 4, apoE3/5 (patient). **b** ApoE genotype examined by a restriction fragment length polymorphism (RFLP). PCR-amplified DNA of *APOE*, including codons 112 and 158, was digested with *HhaI*. Lane 1, DNA size marker (20-bp

DNA ladder); Lane 2, ε3/3 (wild type); Lane 3, ε3/3 (patient); Lane 4, ε2/2; Lane 5, ε3/4. **c** Sequence analysis of PCR-amplified DNA of *APOE*. Both GAG (glutamine; wild type) and AAG (lysine) were observed at codon 3 in exon 3 of *apoE*, indicating that the apoE (Glu3Lys) alleles were heterozygous

Discussion

ApoE5 (Glu3Lys) is involved in hyperlipidemia, despite having twofold greater LDL receptor-binding activity than that of normal apoE3. To explain this paradox, it is hypothesized that the enhanced metabolism of apoE5-containing lipoproteins results in hyperlipoproteinemia via the down-regulation of LDL receptors [2, 3]; however, the precise mechanism is still unclear. A few cases of LPG associated with apoE5 (Glu3Lys) have been reported, but apoE variants specific to LPG, e.g., apoE-Chicago [4] and apoE-Sendai [5], were also detected in these cases, and the specific role of apoE (Glu3Lys) is unknown.

With respect to the onset of FSGS, dyslipidemia [7] and apoE abnormalities [8] also influence the foamy change of macrophages and mesangial cells, although various factors associated with podocyte injury are important. The role of macrophages in FSGS with hyperlipidemia have been demonstrated experimentally [9] and clinically [10] by Saito and coworkers. In the current case, it is noteworthy that apoE5 (Glu3Lys) results from a single mutation in *APOE*. Since apoE5 (Glu3Lys) is involved in hyperlipoproteinemia [2, 3], the mechanism via lipid abnormalities derived from apoE5 as described above may play a pivotal role in the development of FSGS.

The patient was previously treated for diabetes mellitus by a local practitioner, but the diabetic condition was mild, as evidenced by the relatively low blood sugar and HbA1c levels and lack of retinopathy and neuropathy. In addition, MGUS was diagnosed by immunoelectrophoresis and bone marrow aspiration, but characteristic findings of MGUS-related nephropathy were not observed by renal biopsy. Therefore, hyperlipoproteinemia related to apoE5 (Glu3Lys) may be involved in the renal injury in this case.

ApoE combined with triglycerides and cholesterol forms lipoproteins and plays a central role in lipoprotein metabolism [11]. Homozygotic E2 glomerulopathy and LPG are representative renal diseases induced by *apoE* mutations, e.g., homozygous E2 (Arg158Cys) [12] and various novel heterozygous apoE variants [13], respectively. The frequency of apoE5 is very low [1], and there are no reports of kidney disorders related to apoE5, except a few cases of LPG caused by a combination of mutations specific to LPG [4, 5]. Although type III hyperlipoproteinemia or intraglomerular lipoprotein thrombi for the diagnosis of LPG was not observed in this case, the risk of hyperlipidemia due to apoE5 may induce various kinds of kidney diseases, similar to other apoE mutations.

In conclusion, we presented a case of FSGS with apoE5 (Glu3Lys), a rare isoform of apoE. The patient's apoE phenotype was apoE5/3. This is the first case report of the development of nephropathies in a patient who had apoE5 (Glu3Lys), but no other apoE mutations. It is possible that the apoE5

(Glu3Lys) allele plays a pivotal role in the development of FSGS induced by dyslipidemia. However, the precise mechanisms have not been elucidated, and the accumulation of more cases of kidney diseases with spoE5 (Glu3Lys) is required.

Funding None.

Compliance with ethical standards

Conflict of interest All the authors declare that there are no competing interests.

Human and animal rights statement 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 commencement of the phenotype and gene analyses of apoE.

References

1. Matsunaga A, Sasaki J, Moriyama K, et al. Population frequency of apolipoprotein E5 (Glu3→Lys) and E7 (Glu244→Lys, Glu245→Lys) variants in western Japan. *Clin Genet*. 1995;48:93–9.
2. Dong LM, Yamamura T, Yamamoto A. Enhanced binding activity of an apolipoprotein E mutant, APO E5, to LDL receptors on human fibroblasts. *Biochem Biophys Res Commun*. 1990;168:409–14.
3. Dong LM, Yamamura T, Tajima S, Yamamoto A. Site-directed mutagenesis of an apolipoprotein E mutant, apo E5(Glu3→Lys) and its binding to low density lipoprotein receptors. *Biochem Biophys Res Commun*. 1992;187:1180–6.
4. Koder H, Mizutani Y, Sugiyama S, et al. A case of lipoprotein glomerulopathy with ApoE Chicago and ApoE (Glu3Lys) treated with fenofibrate. *Case Rep Nephrol Dial*. 2017;7(2):112–20.
5. Takasaki S, Matsunaga A, Joh K, Saito T. A case of lipoprotein glomerulopathy with a rare apolipoprotein E isoform combined with neurofibromatosis type I. *CEN Case Rep*. 2018;7(1):127–31.
6. Meyrier A. Mechanisms of disease: focal segmental glomerulosclerosis. *Nat Clin Pract Nephrol*. 2005;1:44–54.
7. Diamond JR, Karnovsky MJ. Focal and segmental glomerulosclerosis: analogies to atherosclerosis. *Kidney Int*. 1988;33:917–24.
8. Bruschi M, Catarsi P, Candiano G, et al. Apolipoprotein E in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. *Kidney Int*. 2003;63:686–95.
9. Saito T, Atkins RC. Contribution of mononuclear leucocytes to the progression of experimental focal glomerular sclerosis. *Kidney Int*. 1990;37:1076–83.
10. Saito T, Ootaka T, Sato H, et al. Participation of macrophages in segmental endocapillary proliferation preceding focal glomerular sclerosis. *J Pathol*. 1993;170:179–85.
11. 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.
12. Kawanishi K, Sawada A, Ochi A, et al. Glomerulopathy with homozygous apolipoprotein E2: A report of three cases and review of the literature. *Case Rep Nephrol Urol*. 2013;3:128–35.
13. 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.