A case of lipoprotein glomerulopathy with a rare apolipoprotein E isoform combined with neurofibromatosis type I

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Abstract

Lipoprotein glomerulopathy (LPG) is a rare inherited disease characterized by histopathological features of lipoprotein thrombi in dilated glomerular capillaries and type III like hyperlipoproteinemia with heterozygous mutation of the apolipoprotein (apo) E gene. We herein present the case of a 50-year-old woman with LPG complicated by neurofibromatosis type 1 (NF1). To the best of our knowledge, this is the first report of a case of LPG complicated by NF1. On the other hand, she had not only a heterozygous apoE-Sendai mutation, which is one of the most frequent apoE variants in LPG patients, but also a rare isoform of ApoE5 (Glu3Lys). Although apoE mutation has been recognized as having a principal role in the pathogenesis of LPG, some other factors are assumed to be present in the pathogenesis of LPG, because many asymptomatic carriers of apoE variants are recognized. The coexistence of NF1 or apoE5 (Glu3Lys) allele might play a role as an additional factor in the development of LPG.

Keywords ApoE5 · ApoE-Sendai · Lipoprotein glomerulopathy · Neurofibromatosis type I

Introduction

Lipoprotein glomerulopathy (LPG) is a rare inherited disease characterized by histopathological features of lipoprotein thrombi in dilated glomerular capillaries and type III like hyperlipoproteinemia (HLP) with heterozygous mutation of the apolipoprotein E gene (*APOE*) [1]. On the other hand, neurofibromatosis type 1 (NF1) is an autosomal-dominant disease, characterized by multiple neurofibromas in the skin and skin pigmentation, the so-called café-au-lait spots. No cases of LPG with NF1 have been reported previously. We report herein a case with these two rare diseases, LPG and NF1, along with a rare combination of heterozygous apolipoprotein (apo) isoform E5 (Glu3Lys) and apoE-Sendai.

Case report

A 50-year-old Japanese woman was admitted to our hospital with leg edema. She had been diagnosed with NF1 in childhood, based on clinical signs of multiple subcutaneous nodules, and had been receiving treatment with hypertension for 2 years. Proteinuria had been detected at a routine medical check-up over 10 years earlier. Of her three sisters, one sister had also been diagnosed with NF1, but there was no family history of renal diseases such as nephrotic syndrome or renal failure, and lipidemia for which the antihyperlipidemic therapy is needed including the younger sister with NF1. On admission, height was 151 cm, weight was 56 kg, blood pressure was 128/68 mmHg, and heart rate was 84 beats/min. Physical examination revealed no particular abnormalities other than pitting edema of the lower extremities and numerous subcutaneous nodules on the face, chest, abdomen, and back, and extremities (Fig. 1a). Laboratory findings were as follows: white blood cell count 4200/μL (neutrophils 59.4%; lymphocytes 27.0%; eosinophils 4.8%); hemoglobin 10.4 g/dL; platelet count 295,000/μL; total protein 6.5 g/dL; albumin 3.7 g/dL; hemoglobin A1c 5.7%; serum creatinine 0.81 mg/dL; total cholesterol 299 mg/dL; triglycerides 448 mg/dL; and high-density lipoprotein

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Fig. 1 **a** Cutaneous and subcutaneous nodules on the back. **b** Light microscopic findings of the renal biopsy. Lipoprotein thrombi (arrow) are observed within the capillaries. **c, d** Electron microscopic findings show intra-capillary lipoprotein thrombus-like substances

cholesterol 39 mg/dL. Liver function and total complement were within normal ranges. Results for anti-nuclear antibody were negative. The serum apoE level was 18.4 mg/dL (normal range 2.7–4.3 mg/dL). Lipoprotein analysis using agarose gel electrophoresis showed a broad beta pattern characteristic of type III HLP. Urine protein excretion was 3.07 g/gCr.

Pathological findings

On light microscopy, biopsy specimens of the left kidney contained 28 glomeruli, 7 and 8 of which showed global and segmental sclerosis, respectively. Focal and segmental mesangial cell proliferation and adhesions were identified, whereas endocapillary proliferation with macrophagic infiltration and crescents were not observed. Fifty-seven percent of glomeruli showed marked dilatation of the capillary lumen in the glomeruli filled by a pale-stained substance, which is a characteristic feature of lipoprotein thrombi (Fig. 1b). Electron micrographs showed the thrombus-like substances in the glomerular capillaries, which were composed of vacuoles of various sizes (Fig. 1c, d). The pathological findings corresponded to lipoprotein glomerulopathy.

Phenotype and DNA sequence analysis of apoE

Serum apoE phenotypes were analyzed using isoelectric focusing polyacrylamide gel electrophoresis (IEF), as previously described [2, 3]. As compared to apoE3/3 from a wild-type control, the patient had phenotype apoE2/5 (Fig. 2a).

Sequencing of *APOE* DNA was performed as follows. Three fragments of genomic DNA containing all coding sequence of mature APOE were amplified by polymerase chain reaction with the following primers for *APOE*: 5'-GCTTTCCAAGTGATTAAACCGACT-3' and 5'-AGAGCTAAAGCCAGGAGTCAG-3' for exon 3; and 5'-CCTCTTGGGTCTCTCTGGCT-3', 5'-CTGCTCCTTCACCTCGTCCA-3', 5'-GCAGTACCGCGGCGAGGTGCAGG-3' and 5'-GATCGTGCCACTGCACTCTA-3' for exon 4. Amplified DNA fragments were purified using a PCR purification kit (Qiagen, Germany) and directly sequenced using a Genetic Analyzer 3130xl DNA sequencer (Thermo Fisher Scientific) with a BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific). A substitution of proline for arginine was identified in codon 145 of *APOE* (Fig. 2b). This mutation was heterozygous and was consistent with the apoE-Sendai

Fig. 2 **a** ApoE phenotype determined by isoelectric focusing polyacrylamide gel electrophoresis (IEF). Lane 1, apoE2/5 (patient); Lane 2, apoE2/2; Lane 3, apoE3/3 (wild type); Lane 4, apoE4/4. **b**, **c** Sequence analysis of PCR-amplified DNA of the apoE gene. **b** Both the sequence CGT (arginine; wild type) and the sequence CCT (proline) are observed at codon 145 in exon 4 of the apoE gene, indicat-

ing that the substituted mutation is heterozygous. Substitution of proline for arginine in this site is consistent with apoE-Sendai mutation. **c** Both the sequence GAG (glutamine; wild type) and the sequence AAG (lysine) are observed at codon 3 in exon 3 of the apoE gene, indicating that the apoE (Glu3Lys) alleles are heterozygous

mutation, one of the most frequent apoE variants in LPG patients. The patient also showed heterozygous apoE5 (Glu3Lys), a rare apoE isoform (Fig. 2c). Because apoE-Sendai is known to correspond to apoE phenotype E2 on IEF, apoE-Sendai mutation and apoE5 (Glu3Lys) seemed to be at each other's alleles. These findings indicate that this patient was a compound heterozygote for apoE-Sendai and apoE5 (Glu3Lys).

Clinical course

On the basis of kidney biopsy findings and phenotype and DNA sequence analyses of *APOE*, we diagnosed lipoprotein glomerulopathy. After antihyperlipidemic treatment with bezafibrate at 200 mg/day, urinary protein excretion gradually decreased to almost negative. Although serum cholesterol and triglyceride levels reduced to within normal ranges, serum apoE level decreased slightly (11.6 mg/dL) and remained above the normal range.

Discussion

LPG is an inherited disease first reported by Saito et al. [1], characterized by histopathological features such as dilation of the glomerular capillaries with huge accumulations of lipoprotein-rich material called lipoprotein thrombi. Fifteen novel apoE variants associated with LPG have been identified so far [4]. Although apoE mutation has been recognized as playing a principal role in the pathogenesis of LPG, some other factors have been inferred to be present in the

pathogenesis of LPG, because many asymptomatic carriers of apoE variants have been recognized [4].

ApoE is known to have three rare isoforms of apoE1, apoE5, and apoE7, besides the three major apoE isoforms of E3 (wild type), E2, and E4. To the best of our knowledge, only two cases of LPG with apoE5 (Glu3Lys) have been reported [5, 6] and these are confirmed to have apoE-Chicago by Kodera [5] (Table 1). Accordingly, this is the first report to describe a case of LPG with the combination of apoE-Sendai and apoE5 (Glu3Lys). Although apoE-Chicago [7] and apoE-Sendai [2] are the representative mutations in LPG indicating phenotype E2 on IEF, apoE-Chicago, if apoE5 (Glu3Lys) exists on the same allele, seems to be expressed as phenotype E4, probably by the offset of electrical charge [5]. Few reports have described apoE5, so its prevalence, lipid profile, and associations with disease remain poorly understood. The prevalence of apoE5 has been reported as 0.2% (E3, 78.6%; E4, 13.5%; E2, 7.5%), and individuals with apoE5 develop type II HLP [8] and vulnerability to atherosclerosis due to hypercholesterolemia [9]. The pathogenic mechanisms underlying HLP in patients with apoE5 (Glu3Lys) are presumed to involve downregulation of low-density lipoprotein (LDL) receptors due to high uptake of lipoproteins containing this apoE mutant [10, 11]. Sam et al. [7] demonstrated enhanced binding of apoE-Chicago to glomerular capillaries in a patient with LPG. On the basis of these findings, Kodera et al. [5] speculated that the binding capacity of apoE to glomerular capillaries was altered by the coexistence of apoE-Chicago and apoE5 (Glu3Lys) on the same allele (Case 1 and 3 in Table 1). In the present case, however, apoE-Sendai and

Table 1 ApoE5 (Glu3Lys) association with LPG in the literature

	Age and sex	References	E5 (Glu3Lys)	E2 Chicago (Arg-147Pro)	E2 Sendai (Arg-145Pro)	Classical E2 (Arg158Cys)	apoE phenotype by IEF	apoE mutations and their locations in each allele	Onset of LPG
1	51 years, F	Kodera et al. [5]	○	○	–	–	E3/4	---- E5—Chicago---	○
2	76 years, F	(Mother of case 1) [5]	○	○	–	–	E4/4	--E4--- --E5—Chicago---	–
3	42 years, M	Miyata et al. [6] Re-analyzed by Kodera et al. [5]	○	○	–	○	E2/4	--E2--- --E5—Chicago---	○
4	52 years, F	Present case	○	–	○	–	E2/5	-----Sendai— E5-----	○

apoE5 (Glu3Lys) were at different alleles. Therefore, apoE5 (Glu3Lys) may be involved in the onset of LPG, whether it exists on the same alleles of LPG-related apoE variants or not. On the other hand, Case 2 in Table 1, the mother of Case 1, showed both apoE-Chicago and apoE5 (Glu3Lys) as in Case 1, but nevertheless did not develop LPG. She had an isoform apoE4 on the other allele unlike Case 1. Although it is possible that apoE5 (Glu3Lys), which causes dyslipidemia, contributes to the onset of LPG as an additional factor to LPG-related apoE mutations, the mechanisms of onset for LPG might be more intricate.

NF1 is a rare genetic multi-system disorder, and patients with NF1 have multiple neurofibromas in the skin as a major symptom, as well as bone, eye, adrenal, and gastrointestinal lesions. In NF1, renal involvement is relatively rare. To the best of our knowledge, 12 cases of NF1 associated with glomerular disease have been reported (membranous glomerulopathy, 4 cases; focal segmental glomerulosclerosis (FSGS), 3 cases; IgA nephropathy, 2 cases; minimal-change nephrotic syndrome, 2 cases; and idiopathic nephrotic syndrome, 1 case) [12, 13], while no reports have described NF1 complicated by LPG. Regarding the association between FSGS and NF1, Afshinnia et al. [14] speculated that the decrease in activity of neurofibromin, the product of the NF1 gene, may induce the activation of mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) signaling pathways through the upregulation of Ras proteins. In addition, Koshikawa et al. [15] demonstrated enhanced phosphorylation of p38 MAPK in glomeruli in models of puromycin aminonucleoside and mouse adriamycin nephropathy, and Gödel et al. [16] suggested that mTOR signaling plays a role in podocyte homeostasis. These findings suggest the association of NF1 with glomerular diseases. The decrease in activity of neurofibromin in NF1 might also be associated with LPG which is a glomerular disease, and contribute to the development of LPG as an additional factor. Ito and his colleagues [17] generated

mice with the pathological and serological features of human LPG by apoE and Fc receptor gamma chain double-knockout. Basic studies including experiments with genetically modified mice may reveal the mechanism of the development of LPG and the association with neurofibromin. Unfortunately, we do not perform that kind of experiment, so it is difficult to explain the precise relationship between LPG and NF1 so far.

While the NF1 gene, which is the causative disease gene in most NF1 cases, maps to the long arm of chromosome 17, a few cases with NF1 carry mutation in chromosome 19q13.2 [18]. On the other hand, *APOE* is also located on chromosome 19q13.2. Although we have not performed gene analysis regarding NF1 in our patient, the coexistence of LPG and NF1 might be related to the occurrence of mutations at close position. Since both diseases are associated with genetic abnormalities as described above, it may be necessary to examine the apoE gene abnormality related to LPG when we find NF1 with hyperlipidemia.

In conclusion, we have presented a case of LPG with apoE-Sendai and apoE5 (Glu3Lys), a rare isoform of apoE. Additionally, this is the first report of a case of LPG complicated by NF1. It is possible that the existence of the apoE5 (Glu3Lys) allele or NF1 may play a pivotal role as an additional factor in the development of LPG induced by apoE-Sendai, but the precise mechanisms have not been elucidated. Accumulation of more cases of LPG with the ApoE5 (Glu3Lys) allele or NF1 is required.

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Compliance with ethical standards

Conflict of interest All the authors have declared no competing interest.

Research involving human participants and/or animals 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 phenotype and gene analyses for apoE.

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