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Case Report

Discontinuation of LDL apheresis with evolocumab in an FH patient with a duplication of exon 2–6 in the *LDLR* gene

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ABSTRACT

We report here a familial hypercholesterolemia (FH) patient with a rare mutation, exon 2–6 duplication in the low-density lipoprotein (LDL) receptor gene, who had received LDL apheresis with drug treatment for 15 years. We added evolocumab (proprotein convertase subtilisin/kexin type 9 inhibitor) 140 mg bi-weekly to the treatment, and checked lipid profiles [LDL cholesterol, lipoprotein(a), malondialdehyde-modified LDL, etc.] for 34 weeks. Evolocumab enabled the patient to discontinue LDL apheresis and decrease the dose of statin. We demonstrate that evolocumab contributed to the management of atherogenic lipoproteins in an FH patient with exon 2–6 duplication as an alternative to LDL apheresis. <**Learning objective:** LDL apheresis has been the last therapeutic tool for FH patients, however, the treatment is invasive and time consuming. FH patients show various clinical presentations and different responses to medication depending on their genetic mutations. In this severe heterozygous FH patient which seemed to be homozygous FH, we explored various lipid profiles and assessed the treatment when altering the treatment from LDL apheresis to evolocumab, moreover decreasing the dose of statin.>

Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors were found to be beneficial for reducing low-density lipoprotein cholesterol (LDL-C). In the ODYSSEY ESCAPE trial, LDL apheresis was discontinued in 63.4% of patients on alirocumab, and 92.7% avoided at least half of the apheresis treatments [1]. Familial hypercholesterolemia (FH) is an autosomal dominant disease that causes severe elevations of LDL-C as a result of mutations in the genes encoding LDL receptor (LDLR), apolipoprotein B (Apo B), and PCSK9. FH is usually caused by mutations in the *LDLR* gene, and most of these are point mutations. FH patients with rare mutations, such as duplication or deletion as identified by the multiplex ligation-dependent probe amplification (MLPA) method, tend to fail to achieve their target LDL-C levels, even with the use of various cholesterol-lowering drugs [2,3]. Lehrman and Goldstein reported a reference case in 1987, even though their case, FH295, was a compound heterozygote [3]. They found that only 30–50% binding activity of the LDLR derived from the paternal allele with a duplication exon 2–8 functioned properly. The RUTHERFORD-2 trial suggested that the response to evolocumab may be unrelated to the underlying genetic mutation in heterozygous FH [4]. We diagnosed this patient genetically, and observed the effects of evolocumab. Various lipid profiles were also examined for 34 weeks, when LDL apheresis treatment was switched to evolocumab treatment and the dose of statin was decreased.

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Case report

A 65-year-old woman had been diagnosed with xanthelasma and hypercholesterolemia at age 38 years, but was not diagnosed with FH at that time. At age 50 years, she underwent percutaneous coronary intervention (PCI) to the right coronary artery (RCA) for unstable angina pectoris. At this point, she was diagnosed as having FH according to the Simon Broome criteria, since she presented a high level of total cholesterol (TC) >570 mg/dL and tendon xanthomas. At age 53 years, coronary angiography showed multivessel stenosis with 99% stenosis in the RCA and total occlusion of the left anterior descending artery. She underwent PCI to the RCA again (Fig. 1A). She experienced cerebral infarction with severe stenosis of the right internal carotid artery (Fig. 1B), and LDL apheresis was introduced along with drug treatment. At age 61 years, the reduction in the cholesterol level was deemed to be insufficient, and her medication was increased to rosuvastatin 20 mg/d and ezetimibe 10 mg/d. Pertinent findings in the physical examination included a corneal arcus and thickening of the Achilles tendon (23 and 24 mm thick on the right and left sides) (Fig. 1C). She had a family history of cardiovascular events (her father had died of myocardial infarction at age 45 years) and her son had been diagnosed with hypercholesterolemia. At age 65 years, she presented to our department with high levels of LDL-C despite ongoing treatment with LDL apheresis, and strongly insisted on reducing the amount of medication. This study was approved by the Independent Review Board of Hakujyuji Hospital (#N84) and she gave her written informed consent to participate. DNA analysis was performed by the National Cerebral and Cardiovascular Center according to the protocol approved by the ethics committee (M17-56).

Methods and results

Blood samples were obtained immediately before and after LDL apheresis (weeks 0, 2, 4, and 6), and biweekly before evolocumab administration (weeks 2–30 and 34) (Supplemental Methods).

Baseline concentrations were shown as the mean of lipid profiles before apheresis for the previous 12 months (Fig. 2). To identify the effect of LDL apheresis, lipid profiles pre- and post-apheresis were measured at week 0. From week 0 to 6, LDL apheresis was performed bi-weekly, and evolocumab was administered bi-weekly from week 2. At week 4, this combined treatment gave LDL-C of 71 mg/dL at pre-apheresis, and 9 mg/dL at post-apheresis (87% reduction). At week 6 and 8, LDL-C levels before apheresis were similar to those at week 4 (54 mg/dL and

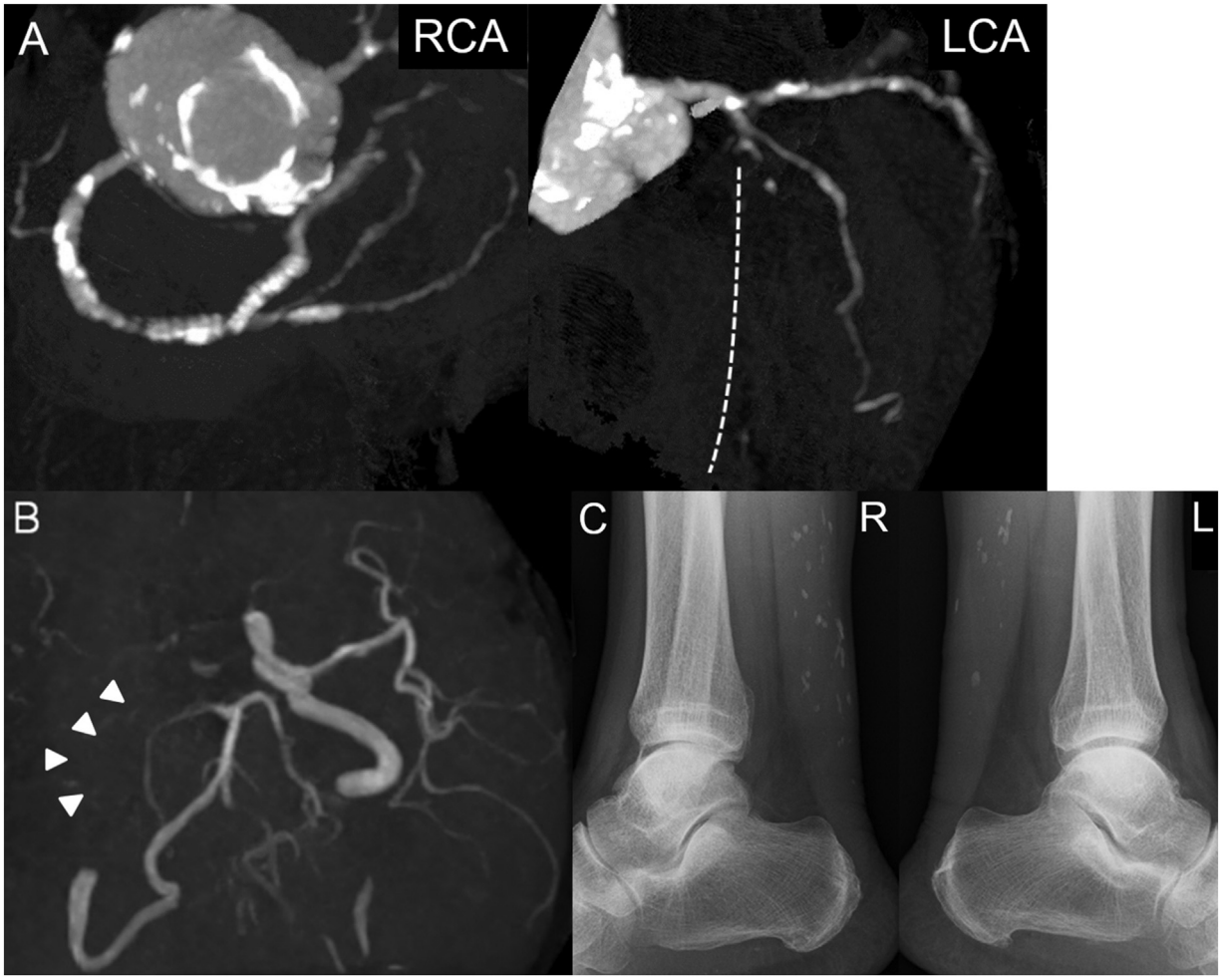

Fig. 1. (A) Coronary computed tomography angiography shows multiple calcified plaques, residual stenoses with right coronary artery after PCI, and an occluded left anterior descending coronary artery (broken line). (B) Magnetic resonance angiogram shows an occluded right internal carotid artery at the origin. (C) Radiography shows severe thickening of the bilateral Achilles tendons [left side: 24 mm, right side: 23 mm].
L, left side; R, right side; LCA, left coronary artery; RCA, right coronary artery; PCI, percutaneous coronary intervention.

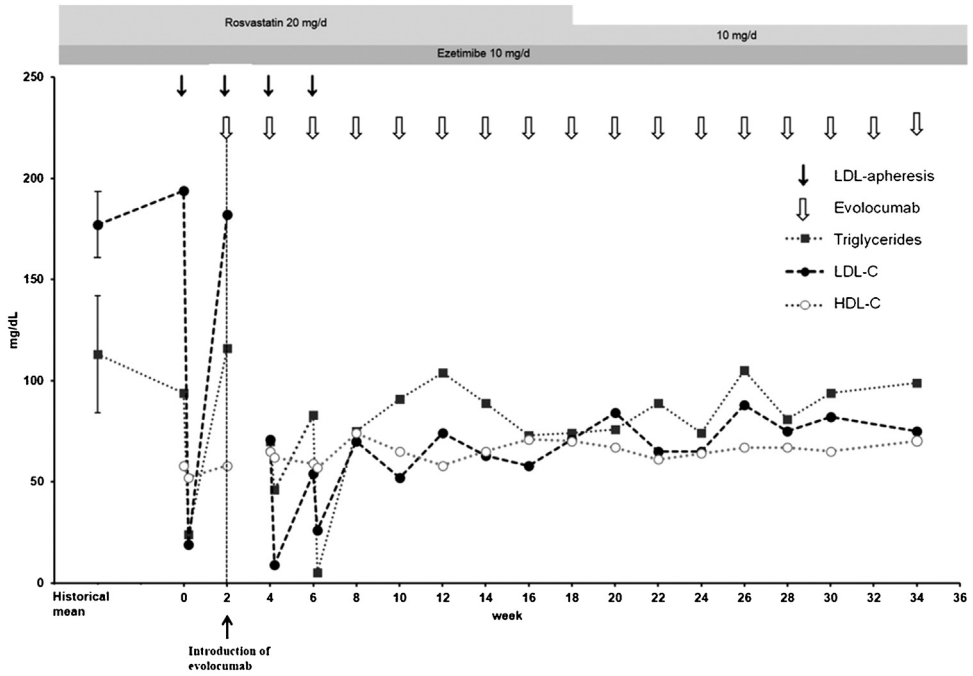

Fig. 2. Treatments and changes in lipid profiles compared to the historical mean at pre-apheresis. Lipid profiles are for LDL-C, HDL-C, and triglycerides. The bar graph shows when rosuvastatin was reduced from 20 mg/d to 10 mg/d at week 18.
LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

70 mg/dL at pre-apheresis, respectively). During weeks 10-18, the LDL-C remained 64 ± 4 mg/dL without LDL apheresis. Furthermore, the dose of rosuvastatin was decreased to 10 mg/d in week 18, however, LDL-C could not be maintained <70 mg/dL (Table 1) [5]. High-density lipoprotein cholesterol (HDL-C) was increased from 52 mg/dL to 58 mg/dL at pre-apheresis (week 2), and increased to 66 ± 4 mg/dL (weeks 10–34). While LDL-C significantly increased by 16% ($p = 0.038$) with the reduction of statin, there were no differences in HDL-C or triglycerides (Table 1).

PCSK9 and other lipid profiles were measured at each point. Similar to the change in LDL-C, there were increases in remnant-like lipoprotein particles cholesterol (RLP-C), lipoprotein(a) [Lp(a)], malondialdehyde-modified LDL (MDA-LDL), Apo B, and apolipoprotein A1 (Apo A1) during weeks 0–2 (from post-apheresis to pre-apheresis). Cholesterol efflux capacity was increased during evolocumab treatment. Cholesterol efflux capacity after the reduction of statin (weeks 20–34; 21 ± 2%) was almost the same as before this reduction (weeks 10–18; 21 ± 0.6%) (Table 1).

Discussion

We investigated the genetic aspects of this patient who clinically appeared to have homozygous FH. Ultimately, we gave a diagnosis of heterozygous FH with duplication of exon 2–6 in the *LDLR* gene, which was detected by the MLPA method. Chiou and Charng found that the response to statin and ezetimibe differed between patients with large rearrangements or nonsense mutations and those with missense or undetected mutations in the *LDLR* gene [2]. Patients with large rearrangements had higher LDL-C, and treatment with statin or statin plus ezetimibe was less able to lower LDL-C levels to ≤100 mg/dL. Few studies have investigated the relationship between the mutation and its activity, and it was suggested that in patients with an abnormal MLPA pattern, the mutation resulted in a "null-receptor" or a truncated protein that lacked important domains for LDLR function [2]. Since the duplication of exon 2–6 has never been reported, nothing is known about its LDLR activity.

Table 1 Time course of various lipid profiles.

	TC (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	Apo A1 (mg/dL)	Cholesterol efflux capacity (%)	Apo B (mg/dL)	Lp(a) (mg/dL)	RLP-C (mg/dL)	MDA-LDL (U/L)	PCSK9 (ng/ml)
Baseline	251 ± 25	113 ± 29	177 ± 17	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Post-apheresis (week 0)	80	24	19	52	137	17.4	14	<1	1.5	14	325
Pre-apheresis (week 2)	272	116	182	58	152	19.9	144	16	7.3	169	945
Apheresis + evolocumab + rosuvastatin 20 mg (week 4–8)	147± 13	76 ± 7	65 ± 10	66 ± 8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Evolocumab + rosuvastatin 20 mg (week 10–18)	154 ± 4	86 ± 5	64 ± 4	66 ± 2	171 ± 5	21 ± 0.6	65 ± 15	8 ± 6	3 ± 0.2	80± 4.0	7142 ± 115
Evolocumab + rosuvastatin 10 mg (week 20–34)	164 ± 4	85 ± 5	76 ± 3	67 ± 2	181 ± 8	21 ± 2	68 ± 8	11 ± 2	3 ± 0.7	83 ± 14	7046 ± 430

Data shown are mean ± SD.
TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; Lp(a), lipoprotein(a); RLP-C, remnant like particles cholesterol; MDA-LDL, malondialdehyde-modified low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; N.D., not determined.

Our patient also presented not only typical manifestations of FH, such as tendon xanthoma and xanthelasma, but also premature cerebral and myocardial infarction. Moreover, even though the target level for LDL-C was set at <70 mg/dL by the Japan Atherosclerotic Society guidelines in 2017, LDL apheresis combined with statin and ezetimibe was insufficient to maintain ≤100 mg/dL. Within the intervals of apheresis, LDL-C returned to the previous high levels. When LDL apheresis was performed with evolocumab, LDL-C was increased only up to 65 ± 10 mg/dL, thus LDL apheresis could be avoided at week 8 (Table 1). It was presumed that the effect of evolocumab was due to at least one preserved functional allele, even though she had a large arrangement in the *LDLR* gene. This observation was consistent with the RUTHERFORD-2 trial and the ODYSSEY ESCAPE trial [1,4]. PCSK9 concentration was remarkably increased by evolocumab. As Rey et al. have reported, this nearly 7-fold increase seems to reflect the effect of evolocumab [6].

Lp(a) was reduced by only 20–30% using PCSK9 inhibitors, compared with 50–70% by LDL apheresis [7]. We confirmed this reduction of Lp(a) (38% reduction). Increasing evidence suggests that RLP-C and MDA-LDL are also independently associated with coronary artery disease. As described above, this case showed that PCSK9 inhibitors reduced these profiles and maintained the reduced levels, while LDL apheresis only transiently, albeit markedly, lowered these profiles. On the effect of sustained reductions by PCSK9 inhibitors, further studies will be needed.

In this case, HDL-C was reduced by approximately 10% in LDL apheresis, whereas it was increased by approximately 20% with evolocumab. Compared with pre-apheresis, Apo A1 was also increased, while Apo B was reduced by approximately 50% with evolocumab. Some studies have indicated that Apo A1 and Apo B could help to predict cardiovascular disease and mortality in comparison with TC, HDL-C, and LDL-C.

Moreover, recent clinical studies have demonstrated that cholesterol efflux capacity was independently and inversely associated with cardiovascular disease [8]. It was suggested that HDL functionality was more important than its quantity. We also stated that cholesterol efflux capacity might be a novel biomarker and a therapeutic target in FH patients [8]. In this case, cholesterol efflux capacity during evolocumab treatment tended to be higher than during apheresis treatment and correlated with the change in Apo A1. In the JUPITER trial, there was no suggestion that rosuvastatin changes cholesterol efflux capacity [9]. It has not been clarified whether the effects of PCSK9 inhibitors or the discontinuation of apheresis causes these changes. However, our observations suggested that PCSK9 inhibitors might be more effective for managing the lipid profiles and outcomes than LDL apheresis.

With regard to the dose of statins, the Rule of 6 is well known as the log-linear dose-response curve [10]. This can be explained from the finding that statins upregulate PCSK9 synthesis in addition to the synthesis of LDLRs. According to Roth et al., there was no significant difference in the decrease in LDL-C between 10 mg and 80 mg of atorvastatin under PCSK9 inhibitor [5]. Therefore, based on these findings and the patient's desire, the dose of rosuvastatin was decreased to 10 mg. However, in contrast, LDL-C was significantly increased ($p = 0.038$). The dose might be insufficient to maintain LDL-C <70 mg/dL, or the susceptibilities to statins and PCSK9 inhibitors may be different depending on *LDLR* mutations. Further studies will be needed to identify the adequate dose of statin combined with PCSK9 inhibitor.

This is the first report on the identification and treatment of a patient with a duplication of exon 2–6 in the *LDLR* gene. Treatment with evolocumab could make it possible to withdraw LDL apheresis and improve various lipid profiles. As described here, FH patients even with a large duplication in the *LDLR* gene likely benefit from PCSK9 inhibitors.

Disclosures

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jccase.2018.10.005.

References

[1] Moriarty PM, Parhofer KG, Babirak SP, Cornier MA, Duell PB, Hohenstein B, et al. Alirocumab in patients with heterozygous familial hypercholesterolaemia undergoing lipoprotein apheresis: the ODYSSEY ESCAPE trial. Eur Heart J 2016;37:3588–95.
[2] Chiou KR, Charng MJ. Detection of mutations and large rearrangements of the low-density lipoprotein receptor gene in Taiwanese patients with familial hypercholesterolemia. Am J Cardiol 2010;105:1752–8.
[3] Lehrman MA, Goldstein JL, Russell DW, Brown MS. Duplication of seven exons in LDL receptor gene caused by Alu-Alu recombination in a subject with familial hypercholesterolemia. Cell 1987;48:827–35.
[4] Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet 2015;385:331–40.
[5] Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA. Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. N Engl J Med 2012;367:1891–900.
[6] Rey J, Poitiers F, Paehler T, Brunet A, DiCioccio AT, Cannon CP, et al. Relationship between low-density lipoprotein cholesterol, free proprotein convertase subtilisin/kexin type 9, and alirocumab levels after different lipid-lowering strategies. J Am Heart Assoc 2016;5:e003323.
[7] Gordon BR, Kelsey SF, Bilheimer DW, Brown DC, Dau PC, Gotto Jr AM, et al. Treatment of refractory familial hypercholesterolemia by low-density lipoprotein apheresis using an automated dextran sulfate cellulose adsorption system. Am J Cardiol 1992;70:1010–6.
[8] Ogura M, Hori M, Harada-Shiba M. Association between cholesterol efflux capacity and atherosclerotic cardiovascular disease in patients with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2016;36:181–8.
[9] Khera AV, Demler OV, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, et al. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: an analysis from the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). Circulation 2017;135:2494–504.
[10] Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol 2003;92:152–60.