

Review

Autosomal Recessive Hypercholesterolemia: A Mild Phenotype of Familial Hypercholesterolemia: Insight from the Kinetic Study using Stable Isotope and Animal Studies

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Autosomal recessive hypercholesterolemia (ARH) is an extremely rare inherited disorder, the cause of which is mutations in the low-density lipoprotein (LDL) receptor adaptor protein 1 (LDLRAP1) gene. Only 36 families with 14 different mutations have been reported in the literature to date. The clinical phenotype of ARH is milder than that of homozygous familial hypercholesterolemia (FH) caused by LDL receptor gene mutations. Recently, the lipoprotein metabolism of ARH was investigated in both humans and mice by several investigators, including ourselves. Based on these findings the preserved clearance of LDL receptor-dependent very-LDL (VLDL) may be a possible mechanism underlying the responsiveness to statins and the milder phenotype of ARH. Although ARH has been described as being “recessive,” several studies, including ours, have indicated that a heterozygous carrier status of the LDLRAP1 gene is associated with mild hypercholesterolemia and exacerbates the phenotype of FH resulting from LDL receptor gene mutations. This review summarizes current understanding regarding ARH and its causative gene, LDLRAP1, and attempts to provide new insight into novel pharmacological targets for treating dyslipidemic patients.

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Key words: Autosomal recessive hypercholesterolemia, LDL receptor adaptor protein 1, Familial hypercholesterolemia

Introduction

Familial hypercholesterolemia (FH) is the most common and most severe monogenic hypercholesterolemia, characterized by the excess deposition of cholesterol in tissues that subsequently leads to the development of tendon xanthomas and premature coronary artery disease¹⁻³. The “classical” form of FH exhibits

autosomal dominant inheritance and is caused by gene defects or mutations in the low-density lipoprotein (LDL) receptor¹⁻³. Individuals with two mutations in the LDL receptor gene (homozygous FH) show extremely severe hyper-LDL cholesterol, with an LDL-cholesterol level usually above 400 mg/dL, and frequently develop cutaneous xanthomas, coronary artery disease and aortic valve stenosis in childhood^{1,2}. If the LDL-cholesterol level is not effectively managed, individuals homozygous for FH die prematurely from acute coronary events and heart failure⁴. The frequency of heterozygous FH in the general population is estimated to be approximately 1 in 500, in almost every population worldwide. Recent advances in genetic analyses have enabled clinicians to diagnose

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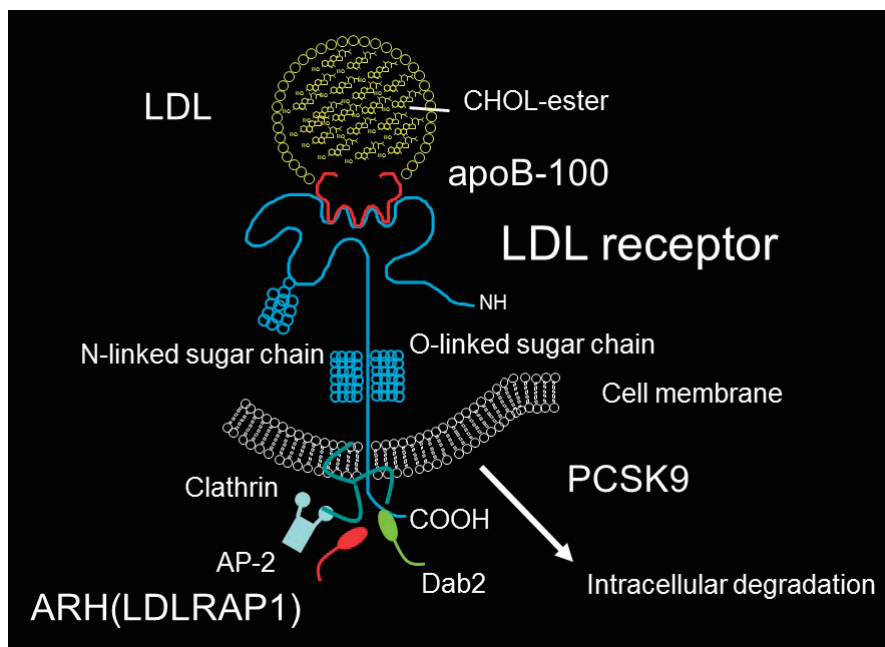


Fig. 1. Metabolic Pathway of LDL.

LDLRAP1 serves as an adaptor protein of LDLR in the cytoplasm in collaboration with clathrin AP-2 and Dab2. This complex internalizes in hepatocytes. The figure is adapted from reference 46.

heterozygous FH more accurately, subsequently revealing a much higher frequency of FH in certain populations, including Afrikaners, Christian Lebanese, Finns and French-Canadians, due to the founder effect¹⁾. Recently, gain-of-function mutations in proprotein convertase subtilisin/kexin type9 (PCSK9) have been found to cause FH⁵⁾. In addition, we previously identified common PCSK9 E32K gene mutations in Japanese patients with FH and reported an estimated frequency of heterozygous FH as high as 1 in 200 in the Hokuriku district of Japan⁶⁻⁸⁾.

In addition to the autosomal dominant inherited form, Khachadurian *et al.* reported an unusual Lebanese family in which all four offspring showed clinical manifestations of homozygous FH, including severe hypercholesterolemia and large tendon xanthomas, with a recessive form of inheritance⁹⁾. Meanwhile, Harada-Shiba *et al.* first reported a Japanese brother and sister with features resembling those of homozygous FH and a normal LDL receptor activity who responded poorly to medical treatment with cholesterol-lowering therapy in 1992¹⁰⁾. Interestingly, the authors had previously described the process of cholesterol turnover after LDL apheresis using a rebound curve, resulting in a defect in the degradation of cholesterol in the affected patient. This disorder was subsequently referred to as autosomal recessive hypercho-

lesterolemia (ARH) and has become widely recognized as a genotype of FH²⁾. We previously reported the second Japanese family with ARH as a result of the same gene mutation seen in the first reported Japanese family^{11, 12)}. Our patients responded well to statin-based cholesterol-lowering therapy, in stark contrast to that observed in the first reported case in Japan.

In this review, we summarize the current understanding of ARH and causal molecule, LDL receptor adaptor protein 1 (LDLRAP1), focusing on the viewpoint of human and animal kinetic studies.

Role of the LDL Receptor and its Related Proteins

The LDL receptor is best known for its role in the catabolism of endogenous lipoproteins (**Fig. 1**). The first step of LDL catabolism is binding between LDL particles and the ligand-binding domain of the LDL receptor. After the receptor binds to LDL particles, the receptor-ligand complex is internalized into hepatocytes and delivered into endosomes. In the acidic environment in the endosome, the LDL receptor folds back on itself, binding the beta-propeller region of the EGF precursor domain into close apposition to the ligand-binding domain. The lipoprotein-released LDL receptor is then recycled and again

Table 1. Updated Version of Reported Mutations in Patients with ARH

Mutation	Protein	Number of Families	Ethnic Origin	Reference
c.65G>A	p.Trp22X	9	Sardinian/S. Italian	27
		5	Sardinian	28
c.70_71del	p.Gly24ArgfsX9	1	Pakistani	28
c.71delG	p.Gly24AlafsX32	1	American	26
c.71_72insG	p.Gly24fsX10	1	Iranian	26
c.88+702ins2613	Null	1	Lebanese	29
c.89-1G>C	p.Lys30ThrX3	1	Syrian Druze	30
c.89-?_747+?del	Null	1	English	28
c.406C>T	p.Gln136X	4	Turkish/Lebanese	26
c.432_433insA	p.Ala145SerfsX26	5	Sardinian/Scillian	26, 27, 31
c.65G>A + c.432insA	p.Trp22X	1	Sardinian	27
c.459+2T>G	Unknown	1	Mexican	32
c.345-195_459+1298del	Null	1	Spanish	33
c.606dup	p.Leu203fsX18	2	Japanese	10, 11, 34
		1	English	28
c.747+773G>A	p.Trp249fsX63	1	Italian	28

transported to the cell surface. Specific mutations in the LDL receptor as well as its ligand apolipoprotein B-100 reduce the affinity of these molecules, thus resulting in congestion of LDL particles in the circulation¹³. Many other mutations in the LDL receptor affect its internalization^{1, 2}.

ARH patients with loss-of-function mutations in LDLRAP1, which have been reported to be involved in the initiation of LDL receptor internalization, show almost the same abnormalities as FH patients with internalization-defective LDL receptor gene mutations. Hence, several studies, including ours, have shown that binding assays using peripheral lymphocytes from ARH patients may overestimate the LDL receptor activity due to overcounting labeled-LDL particles bound to the LDL receptor on the surface of the lymphocytes^{11, 14}. Although the functions of other adaptor proteins, such as disabled-2 (Dab2) and AP-2 are not fully understood, these proteins may be substrates of LDLRAP1 or share other functions with respect to cell growth or division¹⁵⁻¹⁷.

Clathrin-mediated endocytosis is a crucial process for the internalization of a wide range of receptors, including the LDL receptor. The N-terminal domain of LDLRAP1 contains a phosphotyrosine-binding (PTB) domain, which binds to the internalization sequence (FDNPVY) in the cytoplasmic tail of LDLR¹⁵⁻¹⁸. LDLRAP1 proteins serve as an adaptor for LDL receptor endocytosis in hepatocytes, and deficiencies in this protein result in the decline of LDL-cholesterol catabolism, as seen in patients with homozygous FH. However, it is noteworthy that the func-

tion of the LDL receptor is preserved in cultured skin fibroblasts and defective in transformed lymphocytes derived from subjects with ARH, suggesting the existence of alternate pathways that do not require LDLRAP1 proteins, at least in some cell types^{19, 20}. Such pathway(s) may contribute to the milder phenotype as well as responsiveness to statins observed in cases of ARH.

In addition to its central role in cholesterol metabolism in hepatocytes, LDLRAP1 is also known to be expressed in the kidneys. LDLRAP1 is predominantly expressed in the distal nephrons, where renal outer medullary potassium channels are colocalized. The function of LDLRAP1 in the kidneys has been described as playing a key role in potassium homeostasis, at least in mice²¹. Another article indicated that LDLRAP1 proteins are involved in cell cycle progression, possibly via effects on nuclear membrane formation through their interactions with mitotic proteins²². However, there is no evidence of primary potassium retention, hyperkalemia or premature aging in human ARH subjects. Hence, it can be speculated that LDLRAP1 plays a minor role in extra-lipid metabolism in humans.

Megalin, a member of the LDL receptor family, binds to many ligands in several types of epithelial cells, including those in the renal proximal tubules and thyroid and parathyroid glands. The first FXNPXY motif in the cytoplasmic tail of megalin binds to the PTB domain of LDLRAP1, while the second FXNPXY motif interacts with the PTB domain of Dab-2^{23, 24}.

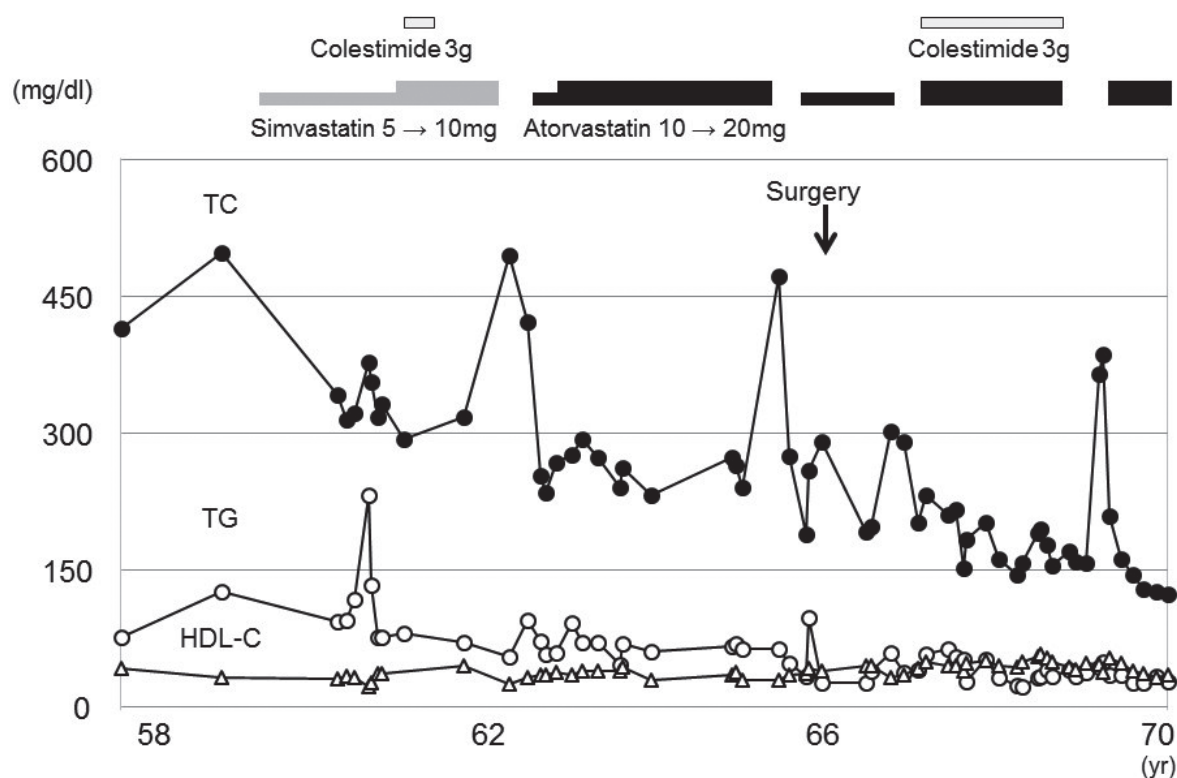


Fig. 2. Clinical course of the ARH patient.

The closed circles indicate TC, the open circles indicate TG and the open triangles indicate HDL-C. The initial serum TC concentration was as high as 513 mg/dL, then decreased to approximately 150 mg/dL after the administration of statin treatment for eight years. Bypass surgery for total occlusion of the bilateral external iliac arteries and left anterior descending artery was conducted at 66 years of age.

Clinical Phenotype of ARH

Khachadurian *et al.* first described the autosomal recessive form of hereditary hypercholesterolemia in 1973⁹⁾. Subsequently, in 1992, Harada-Shiba *et al.* reported the first Japanese family with ARH, including a brother and sister with severe tendon xanthomas born from a consanguineous marriage, with total cholesterol levels of 600 and 533 mg/dL, respectively¹⁰⁾. Following Harada-Shiba's report, several hypercholesterolemic cases involving autosomal recessive inheritance were reported²⁵⁾. In 2001, Garcia *et al.* showed that this disorder is caused by a recessive form of null mutations in the LDLRAP1 gene²⁶⁾. Since then, 36 ARH families with 14 different mutations in the LDLRAP1 gene have been identified (Table 1), mostly from the island of Sardinia, where the frequency of a heterozygous mutation carrier status for the LDLRAP1 gene is estimated to be approximately 1 in 143 individuals²⁷⁻³⁴⁾.

We previously reported the second Japanese family with ARH in 2012¹¹⁾. The proband was a 68-year-

old Japanese man who visited to our hospital with large cutaneous and tendon xanthomas on his fingers and foot that had developed around 10 years of age and a thickness of the Achilles tendon as thick as 26 mm. He had subsequently developed severe coronary artery disease and peripheral artery disease requiring bypass surgery at 66 years of age. His initial serum total cholesterol and triglyceride levels were 513 and 132 mg/dL, respectively, the severity of which ranged between typical homozygous and heterozygous FH caused by LDL receptor gene mutations³⁵⁾. In contrast to that observed in patients with homozygous FH resulting from LDL receptor gene mutations, statin therapy was effective in decreasing his cholesterol level (Fig. 2) and reducing the xanthomas on his hands¹¹⁾.

Clinical Significance of a Heterozygous Mutation Carrier Status of the LDLRAP1 Gene

There are few published data regarding the clinical characteristics of LDLRAP1 heterozygous muta-

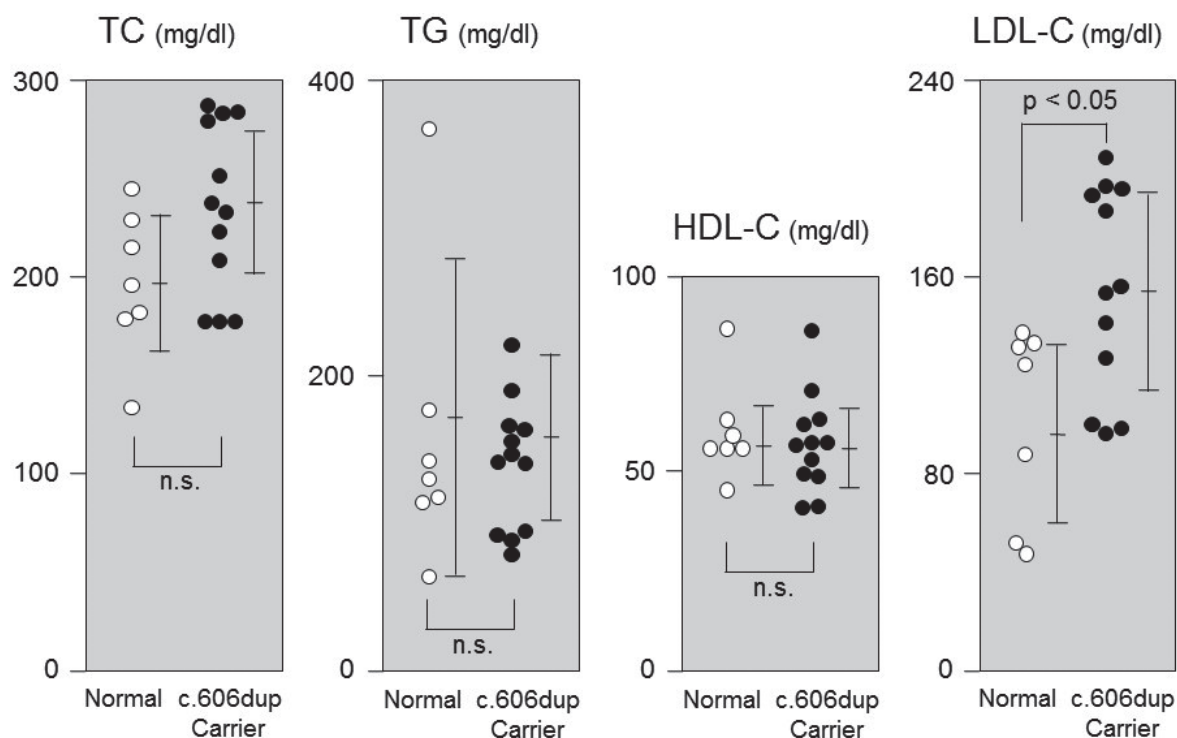


Fig. 3. Impact of the heterozygous mutation in the LDLRAP1 gene on the lipid levels.

The LDL-C levels in c.606dup mutation carriers are significantly higher than those observed in unaffected family members, although there are no significant differences in other parameters.

tion carriers. Our group previously reported that heterozygous c.606dup mutation carriers of the LDLRAP1 gene exhibit significantly higher LDL-cholesterol levels than their normal siblings (154 ± 36 vs. 108 ± 41 mg/dL, respectively) (**Fig. 3**)^{11,36}. Although a controversial report was published later, it is thought that a heterozygous c.167C>T mutation in the LDLRAP1 gene causes the FH phenotype³⁷. Interestingly, additional LDLRAP1 gene mutations exacerbate the phenotype of FH in patients with LDL receptor gene mutations with respect to the LDL-cholesterol levels and severity of xanthomas³⁸. These data indicate that ARH may not be a fully “recessive” disorder, but rather causes mild hyper-LDL cholesterolemia.

Abnormal Lipoprotein Metabolism in Patients with ARH

Because ARH is an extremely rare disorder, little is known about the details of its lipoprotein metabolism. Harada-Shiba *et al.* estimated the fractional catabolic rate (FCR) of the LDL fraction in ARH patients using the two-compartment method following the administration of plasmapheresis therapy. Consequently, the FCR and production rate (PR) of chole-

sterol in the ARH subjects were estimated to be 0.102 pool/day and 19.4 mg/kg/day, respectively, whereas those for patients with homozygous and heterozygous FH due to LDL receptor gene mutations were found to be 0.101 pool/day and 29.2 mg/kg/day and 0.280 pool/day and 21.2 mg/kg/day, respectively¹⁰.

Zuliani G. *et al.* performed an *in vivo* ¹²⁵I-LDL kinetic study in three ARH patients without a genetic diagnosis at that time in addition to normolipidemic controls²⁵. The authors found that the ¹²⁵I-LDL FCR and PR values in the ARH patients were significantly lower (0.19 vs. 0.26 pools/day) and higher (20.7 vs. 14.0 mg/kg/day) than those noted in the normal subjects, respectively. Although these *in vivo* kinetic results reinforce the findings of Harada-Shiba *et al.*, the detailed abnormalities in lipoprotein metabolism, other than the LDL fraction, as well as the mechanism of responsiveness to statins remain unclear.

Jones *et al.* demonstrated that the rate of very-LDL (VLDL) clearance is significantly higher in LDLRAP1 ^{-/-} mice than in LDL receptor ^{-/-} mice, suggesting that VLDL is cleared in hepatocytes via a LDL receptor-dependent pathway in LDLRAP1 ^{-/-} mice (**Fig. 4**)³⁹. Therefore, it can be speculated that the preserved ability of LDL receptor-dependent VLDL

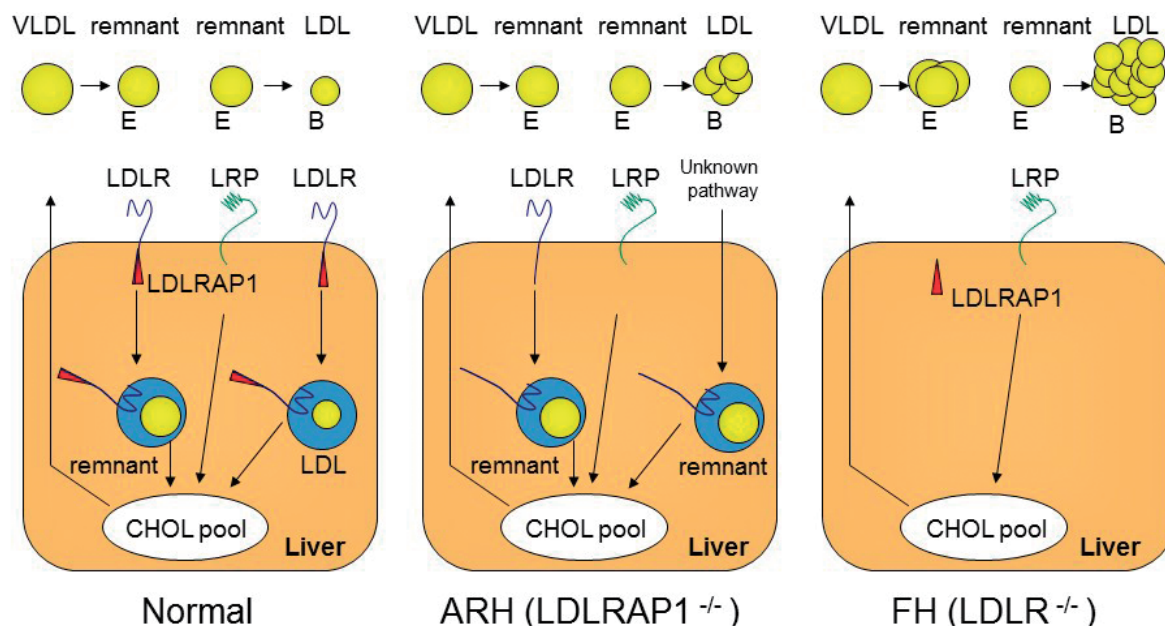


Fig. 4. Schematic figure of the possible mechanism of the mild phenotype in ARH.

VLDL and its remnants may be catabolized via pathway(s) other than those involving LDLR and LDLR-related protein (LRP) in patients with ARH.

clearance is a possible mechanism underlying the responsiveness to statins and the milder phenotype observed in ARH patients.

We performed an apoB kinetic study using a stable isotope in an ARH patient for the first time. Our findings subsequently demonstrated that the FCR values of LDL (0.109 vs. 0.450 pools/day) and VLDL (3.153 vs. 8.408 pools/day) apoB in the ARH patients were decreased by approximately 76% and 62%, respectively, compared with those observed in the control subjects at baseline. Interestingly, statin therapy increased both the LDL and VLDL apoB FCR values by almost the same degree as that seen in the control subjects. On the other hand, removal of the VLDL remnant as well as other remnant fractions of ARH was paradoxically increased in the ARH patients, whereas these pathways were inactive in the normal control subjects¹¹). One possible explanation for this paradoxical acceleration of remnant lipoprotein fractions in the setting of ARH is the existence of another catabolic pathway that is independent of FDNPVY internalization for VLDL and its remnants without LDLRAP1 proteins⁴⁰). In addition, it has been demonstrated that a deficiency of the molecule enhancing affinity between ligands, such as the VLDL remnant and LDLR, accelerates the internalization of the remnants. Hence, if LDLRAP1 serves as an anchor between the VLDL remnant and LDLR, a deficiency

in this protein may result in the acceleration of VLDL remnant catabolism in patients with ARH⁴¹). Another possibility is that unknown pathways exist that are inactivated in the presence of LDLRAP1. This hypothesis appears to be supported by the fact that LDLR is able to transfer such remnants to additional receptors for uptake by hepatocyte when its internalization is impaired. These pathways do not always necessarily occur via LDLR, LDLR-related proteins and/or heparan sulfate proteoglycan⁴²). A possible mechanism underlying the mild phenotype of ARH is summarized in **Fig. 4**. The supposed pathway(s) should contribute to the development of the mild phenotype as well as the responsiveness to statins in cases of ARH.

Postprandial Lipoprotein Metabolism in Patients with ARH

In accordance with the process of internal lipoprotein metabolism, postprandial remnant lipoprotein metabolism, including that of chylomicron and its remnant, appears to be preserved, in contrast to that of FH caused by LDLR mutations, in which postprandial remnant lipoprotein metabolism has been shown to be severely impaired⁴³⁻⁴⁵). We performed an oral fat tolerance test in order to elucidate the clearance of both exogenous and endogenous lipoproteins¹²). Our results suggested that ARH preserves the

ability to clear postprandial remnant lipoproteins, especially chylomicron and its remnant fractions, indicating that alternative pathways catabolizing VLDL and the VLDL remnant also clear chylomicron and its remnant fractions¹²⁾. These advantages in the catabolism of postprandial lipoproteins may also contribute to the milder phenotype and responsiveness to statins noted in patients with ARH.

Conclusions

This review summarizes current understanding regarding ARH and its causative gene, LDLRAP1. Recent advances in genetic diagnoses have made it easier to detect rare inherited disorders, such as ARH⁴⁶⁾. Because the responsiveness to medical cholesterol-lowering therapy and prognosis are better than those for classical FH due to LDL receptor gene mutations, it is important to confirm the differential diagnosis of ARH. Further investigations regarding the function of this adaptor protein may provide new insight into the development of novel pharmacological targets for dyslipidemic patients.

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Conflicts of Interest Disclosure

None.

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