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Editorial

PCSK9 inhibition for autosomal recessive hypercholesterolemia

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Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) inhibition with monoclonal antibodies, either as monotherapy or in combination with other lipid-lowering therapies, has recently emerged as a promising strategy to lower circulating LDL by > 50% in patients with a wide range of dyslipidaemia and cardiovascular risk. In the development program of lipid lowering drugs, it is important to demonstrate efficacy in as a wide range of diagnostic categories as possible. The most severe disorders are of special interest, as this is where the unmet treatment needs are greatest. Homozygous familial hypercholesterolemia (HoFH) is a very severe genetic disorder of lipoprotein metabolism that frequently causes markedly premature cardiovascular morbidity and mortality. HoFH is currently inadequately treated with conventional lipid-lowering medications. The microsomal triglyceride transfer protein inhibitor lomitapide and the apoB-synthesis inhibitor mipomersen were licensed for use in HoFH, and although effective, both agents have significant adverse effects and limited tolerability. There is thus a compelling need to develop alternative, better-tolerated therapies for HoFH.

HoFH results primarily from mutations on both low-density lipoprotein receptor (LDLR) alleles, and rarely from mutations on both alleles of the LDLR adaptor protein 1 (LDLRAP1) [1,2]. This particular disorder is known as Autosomal Recessive Hypercholesterolemia (ARH). Because PCSK9 enhances the intracellular degradation of LDLR, PCSK9 inhibition does not lower plasma low-density lipoprotein cholesterol (LDLC) in HoFH patients with mutations that completely abolish receptor function (*receptor-negative*). However, in carriers of LDLR mutations with reduced but not absent LDLR function (*receptor-defective*), these agents reduce LDLC on average by 25% on top of existing treatments [3–6]. In contrast, patients with ARH lack the LDLRAP1 adaptor allowing LDLR interaction with the clathrin machinery, thereby precluding LDLR endocytosis [2]. Whereas LDLRAP1 is required for LDLR internalization in most cell types (e.g. hepatocytes, lymphocytes), it is not in others (e.g. fibroblasts, endothelial cells). For instance, in the liver, LDLRAP1 plays the adaptor role in hepatocytes whereas Disabled-2 (Dab2) participates in LDLR-mediated LDL uptake in sinusoid endothelial cells as the adaptor [7,8]. It is thus possible that adding PCSK9 inhibitors to statins may protect the LDLR from degradation in cells where LDLR endocytosis is not dependent on the LDLRAP1, allowing an increase in LDLC clearance [9]. It is not known whether ARH patients will respond to PCSK9 inhibitors, and this is examined in the paper published by Rodriguez-Jimenez et al. in this issue of *Atherosclerosis* [10].

Untreated LDL cholesterol levels are on average 50 mg/dL lower in ARH patients than in *receptor-defective* HoFH patients [11]. ARH patients also appear to respond slightly better to statins and ezetimibe that HoFH patients [12]. However, only a small minority of ARH and HoFH can reach LDL-C therapeutic levels with these medications. It has been shown in LDLRAP1 deficient mice that VLDL and remnant lipoproteins are cleared normally, which may partially explain the somewhat milder phenotype and better statin response observed in ARH than in HoFH patients. A handful of ARH patients treated with PCSK9 inhibitors have been reported, and surprisingly, their response to either alirocumab or evolocumab is extremely variable, ranging from +3.5% to −35% changes in LDL-C levels [4,9,13,14]. A simple explanation for this variability would be that distinct LDLRAP1 mutations promote an *adaptor-negative* or an *adaptor-defective* pattern. The patient reported by Rodriguez-Jimenez et al. is interesting in that respect, as his LDLRAP1 mutation affects the translation start codon (AUG > GUG) and results in the synthesis of an N-terminally truncated adaptor protein of 32 kDa *vs*. 35kDa for wild-type [10], contrasting with all the other mutations reported to date that consist in the occurrence of premature stop codons on LDLRAP1 and result in the synthesis of C-terminally truncated proteins or no protein at all [2,9].

As shown previously by our team and others, the lymphocytes of the ARH patient reported by Rodriguez-Jimenez et al. display higher LDLR cell surface expression but show reduced fluorescent LDL uptake than

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Abbreviations: PCSK9, Proprotein Convertase Subtilisin Kexin Type 9; LDLC, low density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; HoFH, homozygous familial hypercholesterolemia; ARH, autosomal recessive hypercholesterolemia; LDLRAP1, LDLR adaptor protein 1; Dab2, Disabled-2

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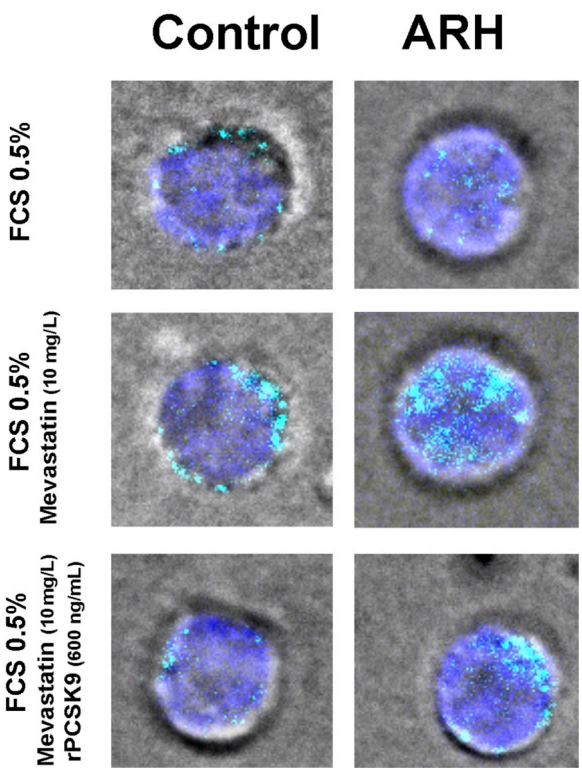

Fig. 1. LDLR expression in control and ARH lymphocytes.
Lymphocytes from one control and one ARH patient were seeded in 96-well plates in RPMI containing 0.5% fetal calf serum (FCS) for 2 h at 37 °C. The culture medium was supplemented with or without mevastatin for 24 h. Recombinant PCSK9-D374Y was added for an additional 4 h in a subset of wells. Lymphocytes were transferred into Millicell EZ slides for the final 2 h of the incubation. Cells were fixed, permeabilized and incubated overnight with a monoclonal antibody against the LDLR. Lymphocytes were incubated with a biotin conjugated goat anti-mouse IgG secondary antibody for 45 min, and Alexa 568-conjugated streptavidin for 15 min in the dark. Slides were mounted in anti-fade reagent containing DAPI to stain nuclei. Representative confocal microscopy images of total LDLR expression are displayed. Nuclei appear in dark blue, and LDLR stacked on the z plane in light blue.

control lymphocytes [9]. Very elegantly, they included in their study the lymphocytes of one HoFH patient carrying a missense mutation located within the sequence motif of the LDLR cytoplasmic domain that directly interacts with LDLRAP1 [10]. These lymphocytes similarly display higher LDLR cell surface expression than control lymphocytes, and show reduced fluorescent LDL uptake when compared with control cells and intriguingly also when compared with ARH lymphocytes, suggesting that LDLRAP1 is not absolutely mandatory for LDL uptake in lymphocytes. Although beyond the scope of that study, it would have been interesting to comparatively assess LDLR cell surface expression and fluorescent LDL uptake before and after statin and/or recombinant PCSK9 treatment in these patients lymphocytes. Compared with controls, ARH lymphocytes always display higher LDLR expression in the absence or presence of statin and/or recombinant PCSK9, as shown in Fig. 1 by confocal microscopy.

Although the ARH patient reported by Rodriguez-Jimenez et al. showed impressive responses to standard lipid lowering treatments, his LDL-C levels dropping from 535 mg/dL down to 146 mg/dL on atorvastatin 80 mg/day plus ezetimibe 10 mg/day, he did not respond to 420 mg/month of evolocumab at all. In contrast, his LDL-C levels dropped down to 87 mg/dL with evolocumab 420 mg injections every fortnight. An increase in the dosing frequency of evolocumab in HoFH patients that enhances treatment efficacy is in line with the very high circulating levels of PCSK9 observed in such patients [6,15]. This does not however explain why the evolocumab monthly dosage did not show any significant LDL-C lowering effect in this patient, as well as in a few HoFH patients with receptor-defective LDLR defects [4,6].

The study by Rodriguez-Jimenez et al. adds fuel to the concept that the higher the extent of residual LDLR function in HoFH or ARH, the more efficacious the response to lipid-lowering medications. It remains to be seen how other factors [e.g. apolipoprotein E genotypes, levels of lipoprotein (a)] modulate the LDL-C lowering efficacy of statins and PCSK9 inhibitors.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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