The CCC Complex COMManDs Control of LDL Cholesterol Levels

Michael F. Keating, Anna C. Calkin

The LDLR (low-density lipoprotein [LDL] receptor) plays a key role in the regulation of plasma LDL cholesterol levels, and loss of function mutations in the LDLR result in familial hypercholesterolemia.[1] After binding of LDL at the cell surface, LDLR is endocytosed via clathrin-coated pits.[2] On release of its cargo, it is then recycled back to the plasma membrane. Alternatively, LDLR can be targeted to the lysosome for degradation via either PCSK9 (proprotein convertase subtilisin/kexin type 9)[3] or IDOL (inducible degrader of the LDLR),[4] in turn reducing LDL uptake. In this issue of *Circulation Research*, Fedoseienko et al[5] demonstrate that LDLR recycling to the cell surface is reliant on an intact CCC (COMMD [copper metabolism MURR1 domain]–CCDC [coiled-coil domain-containing] 22–CCDC93) complex, as well as expression of all COMMD family members. When the integrity of this pathway is compromised, hypercholesterolemia and atherosclerosis ensue.

Article, see p 1648

COMMD 1 is 1 of 10 COMMD family members and was initially identified for its role in regulating copper homeostasis.[6,7] van de Sluis et al provided a link between COMMD1 and copper transport, demonstrating that COMMD1, together with CCDC22 and CCDC93, as well as the WASH (Wiskott–Aldrich syndrome protein and SCAR homologue) complex, regulates endosomal trafficking of the copper transporter, ATP7A (ATPase copper transporting alpha).[8] Subsequent studies by this team demonstrated that this same mechanism is involved in endosomal trafficking of the LDLR[9] (Figure). They reported that deficiency of COMMD1 resulted in impaired LDLR recycling and reduced LDL uptake. Moreover, depletion or mutations in components of the CCC complex, including *Commd1* or *Commd9* or *Ccdc22*, resulted in elevated circulating cholesterol levels in dogs, mice, and humans, respectively.

In the current article, they provide further developments in the understanding of this mechanism regulating endosomal LDLR trafficking, and describe its impact on cardiovascular disease. The authors demonstrate that liver-specific deletion of *Commd6* in mice is associated with elevated plasma total and LDL cholesterol levels, mimicking the effects they previously reported in hepatic *Commd1* knockout mice. Consistent with this, *Commd6* null hepatocytes exhibited reduced total and cell surface expression of not only LDLR but also LRP (LDLR-related protein) 1, which they postulate was due to increased lysosomal degradation as a result of impaired recycling. Interestingly, these mice exhibited no change in their hepatic cholesterol levels, presumably because of a compensatory upregulation of the cholesterol biosynthesis pathway, although this was not assessed.

The authors elegantly use CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9) gene editing to demonstrate that COMMD6 colocalizes with the WASH complex in the endosome in a similar manner to COMMD1 (Figure), as well as with COMMD1 itself. Their studies also highlight the requirement of an intact CCC complex for endosomal trafficking. Specifically, they demonstrate that COMMD6 is required for stability and integrity of the CCC complex because *Commd6* deletion in hepatocytes results in a reduction in the CCC complex components, CCDC22 and CCDC93. Given that all COMMD family members can interact with CCDC22, they extended their studies to examine all COMMD proteins. They demonstrate that COMMD6 and COMMD1 physically interact with each other and that deletion of *Commd6* was associated with attenuation of not only COMMD1 but all family members. Similar effects on COMMD family members were observed with hepatic deletion of *Commd1* and *Commd9* albeit to differing degrees; however, a robust and consistent reduction in CCC components was observed in all *Commd*-deficient models. This demonstrates the dependence of COMMD proteins on their family members; however, why so many COMMD family members exist in the liver given their lack of compensatory upregulation in this setting remains unclear. For example, the authors have previously demonstrated that notch receptor recycling only involves COMMD5 and COMMD9,[10] and they suggest that the COMMD proteins can provide specificity to the endosomal trafficking process. Interestingly, in contrast to *Commd1* or *Commd6* deletion, which almost abolished COMMD6 or COMMD1, respectively, deletion of COMMD9 was associated with only a ≈40% reduction in COMMD1 and COMMD6 protein levels, yet a concomitant increase in total cholesterol and LDL cholesterol levels to that seen with COMMD1 or COMMD6 deletion was still observed. This suggests that even a minor disturbance in the expression of COMMD family members is sufficient to impact on the regulation of cholesterol levels.

In addition to COMMD family members, the authors also disrupt the CCC complex via attenuation of hepatic

From the Lipid Metabolism and Cardiometabolic Disease Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia (M.F.K., A.C.C.); and Central Clinical School, Monash University, Melbourne, Australia (M.F.K., A.C.C.).

Correspondence to Anna C. Calkin, PhD, Lipid Metabolism and Cardiometabolic Disease Laboratory, Baker Heart and Diabetes Institute, 75 Commercial Rd, Melbourne 3004, Australia. E-mail anna.calkin@baker.edu.au

(*Circ Res*. 2018;122:1629-1631.
DOI: 10.1161/CIRCRESAHA.118.313074.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.118.313074

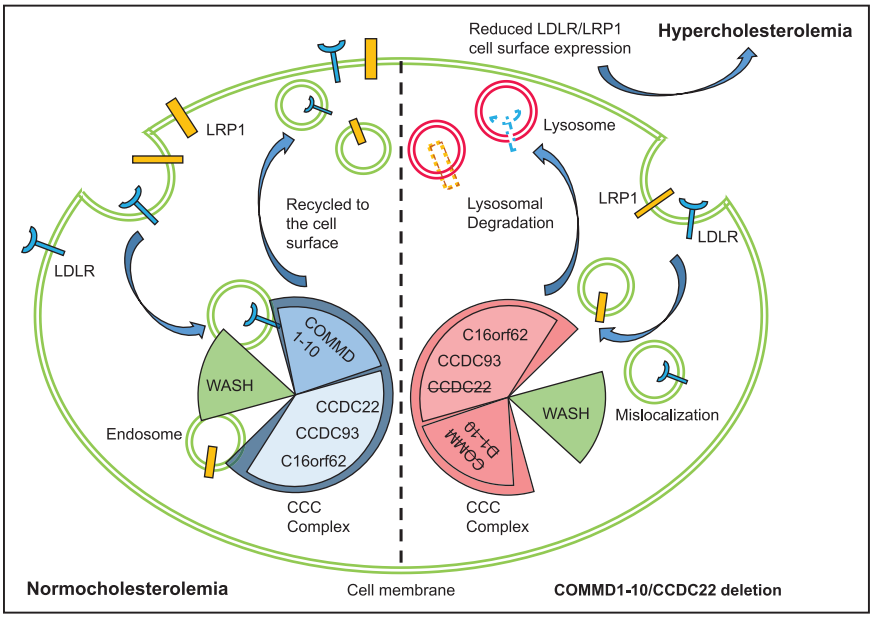

Figure. Hypothesized mechanism of CCC (COMMD [copper metabolism murr1 domain]–CCDC [coiled-coil domain-containing] 22–CCDC93) complex-mediated LDLR (low-density lipoprotein receptor) and LRP1 (low-density lipoprotein receptor–related protein 1) endosomal trafficking. Left, After endocytosis, LDLR/LRP1 is directed to the WASH (Wiskott–Aldrich syndrome protein and SCAR homologue)/CCC complex for recycling to the cell surface. **Right**, Deletion of *Commd1–10* or *Ccdc22* deletion results in improper formation of the WASH/CCC complex leading to mislocalization and enhanced lysosomal degradation. LDLR/LRP1 expression at the cell surface is subsequently diminished, resulting in hypercholesterolemia. C16orf62 indicates chromosome 16 open reading frame 62.

CCDC22 expression in vivo using a CRISPR/Cas9 approach. Remarkably, this resulted in a reduction in all CCC components, including all COMMD proteins except COMMD6. Concomitant hypercholesterolemia was observed in these mice, demonstrating the reliance of an intact CCC core complex for cholesterol regulation. These findings replicate those in humans, demonstrating that mutations in *Ccdc22* are associated with hypercholesterolemia. Furthermore, they highlight the cross-species conservation of this mechanism. This also raises the question of the relative contribution of the different components of the CCC to lipoprotein receptor trafficking and importantly, whether rescue of a single component would be sufficient for restoration of the complex and intact function.

The authors assess the impact of disruption to the CCC complex in an in vivo model of hepatic LDLR insufficiency, mice treated with a gain-of-function *Pcsk9* variant in liver via adeno-associated virus. They observed a marked elevation in total and LDL cholesterol levels and increased apoB48 and apoB100 levels with hepatic *Commd1* deletion in these mice, which they attribute to effects on LRP. Indeed, these findings mimic those observed in *Ldlr/Lrp*-deficient mice.[11] This begs the question, what other lipoprotein receptors are regulated by the CCC complex. Given that COMMD1 binds to the NPxY motif within the LDLR cytoplasmic tail,[9] which is common to other LDLR family members, including VLDLR (very low-density lipoprotein receptor) and apoER2 (apolipoprotein E receptor 2), it would be interesting to investigate the importance of this pathway in the regulation of these receptors. Moreover, the significance of this pathway in other tissues, such as the brain and intestine, would also be of interest. Although this mechanism appears to be independent of PCSK9, IDOL acts in a complimentary but independent manner to degrade the LDLR.[12] Therefore, it would be interesting to examine the functional impact of mutations in the CCC pathway on IDOL-mediated LDLR degradation.

Finally, hepatic *Commd1* deficiency in atherosclerosis-prone apoE3*Leiden mice resulted in a marked elevation in both total cholesterol and triglyceride plasma levels, including LDL and VLDL (very low-density lipoprotein) cholesterol. A concomitant elevation in foam cell-rich atherosclerotic lesions was observed in the aortic root of these mice. This highlights the implications of disruption of this pathway on cardiovascular disease.

In summary, the authors present a range of proteins within the CCC complex that impact on cholesterol regulation in mice. Mutations in CCDC22, which result in a severe developmental disorder, X-linked intellectual disability, have more recently been linked to elevated LDL cholesterol levels in humans, highlighting the cross-species conservation of this regulatory mechanism[9]; however, it remains to be established what the relevance of other members of this pathway is to cholesterol regulation and cardiovascular disease risk in humans. In addition to cholesterol homeostasis, members of the CCC complex have been shown to play a role in copper homeostasis,[7] NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation,[13] notch signaling,[10] HIF (hypoxia-induced factor) 1 regulation, and tumor cell invasion,[14] and mutations have been associated with ectodermal dysplasia in humans.[13] Given its widespread effects, the therapeutic relevance of the CCC complex in the context of cholesterol regulation remains to be determined. Nevertheless, these findings provide valuable insight into the fundamental regulation of a pathway that impacts on LDL cholesterol levels and cardiovascular disease risk.

Acknowledgments

We thank Dr Brian Drew for critical review of the manuscript.

Sources of Funding

A.C. Calkin is funded by a National Heart Foundation of Australia Future Leader Fellowship (100067).

Disclosures

None.

References

1. Goldstein JL, Sobhani MK, Faust JR, Brown MS. Heterozygous familial hypercholesterolemia: failure of normal allele to compensate for mutant allele at a regulated genetic locus. *Cell*. 1976;9:195–203.

2. Anderson RG, Brown MS, Goldstein JL. Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. *Cell*. 1977;10:351–364.
3. Maxwell KN, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc Natl Acad Sci USA*. 2004;101:7100–7105. doi: 10.1073/pnas.0402133101.
4. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science*. 2009;325:100–104. doi: 10.1126/science.1168974.
5. Fedoseienko A, Wijers M, Wolters JC, et al. The COMMD family regulates plasma LDL levels and attenuates atherosclerosis through stabilizing the CCC complex in endosomal LDLR trafficking. *Circ Res*. 2018;122:1648–1660. doi: 10.1161/CIRCRESAHA.117.312004.
6. van De Sluis B, Rothuizen J, Pearson PL, van Oost BA, Wijmenga C. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. *Hum Mol Genet*. 2002;11:165–173.
7. Vonk WI, Bartuzi P, de Bie P, Kloosterhuis N, Wichers CG, Berger R, Haywood S, Klomp LW, Wijmenga C, van de Sluis B. Liver-specific Commd1 knockout mice are susceptible to hepatic copper accumulation. *PLoS One*. 2011;6:e29183. doi: 10.1371/journal.pone.0029183.
8. Phillips-Krawczak CA, Singla A, Starokadomskyy P, et al. COMMD1 is linked to the WASH complex and regulates endosomal trafficking of the copper transporter ATP7A. *Mol Biol Cell*. 2015;26:91–103. doi: 10.1091/mbc.E14-06-1073.
9. Bartuzi P, Billadeau DD, Favier R, et al. CCC- and WASH-mediated endosomal sorting of LDLR is required for normal clearance of circulating LDL. *Nat Commun*. 2016;7:10961. doi: 10.1038/ncomms10961.
10. Li H, Koo Y, Mao X, Sifuentes-Dominguez L, Morris LL, Jia D, Miyata N, Faulkner RA, van Deursen JM, Vooijs M, Billadeau DD, van de Sluis B, Cleaver O, Burstein E. Endosomal sorting of Notch receptors through COMMD9-dependent pathways modulates Notch signaling. *J Cell Biol*. 2015;211:605–617. doi: 10.1083/jcb.201505108.
11. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest*. 1998;101:689–695. doi: 10.1172/JCI1240.
12. Scotti E, Hong C, Yoshinaga Y, Tu Y, Hu Y, Zelcer N, Boyadjian R, de Jong PJ, Young SG, Fong LG, Tontonoz P. Targeted disruption of the idol gene alters cellular regulation of the low-density lipoprotein receptor by sterols and liver x receptor agonists. *Mol Cell Biol*. 2011;31:1885–1893. doi: 10.1128/MCB.01469-10.
13. Starokadomskyy P, Gluck N, Li H, et al. CCDC22 deficiency in humans blunts activation of proinflammatory NF-κB signaling. *J Clin Invest*. 2013;123:2244–2256. doi: 10.1172/JCI66466.
14. van de Sluis B, Mao X, Zhai Y, Groot AJ, Vermeulen JF, van der Wall E, van Diest PJ, Hofker MH, Wijmenga C, Klomp LW, Cho KR, Fearon ER, Vooijs M, Burstein E. COMMD1 disrupts HIF-1alpha/beta dimerization and inhibits human tumor cell invasion. *J Clin Invest*. 2010;120:2119–2130. doi: 10.1172/JCI40583.

KEY WORDS: Editorials ■ animals ■ atherosclerosis ■ humans ■ mice

The CCC Complex COMManDs Control of LDL Cholesterol Levels

Michael F. Keating and Anna C. Calkin

Circ Res. 2018;122:1629-1631
doi: 10.1161/CIRCRESAHA.118.313074
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571