I. Introduction

It has been definitively established that low-density lipoprotein (LDL) cholesterol (LDL-C) is a causal risk factor for atherosclerotic cardiovascular disease (ASCVD). Over the decades, LDL-lowering therapies, including statins, ezetimibe, and proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors have been introduced in clinical settings, and they have contributed to better prognosis. It is of note that the beneficial effects of reducing ASCVD risk appear to be proportional to the absolute degree of reducing LDL-C (Fig. 1). In addition, Mendelian randomization studies, which could be considered as a substitute of randomized controlled trials (RCT), consistently revealed that genetic variations associated with LDL-C was robustly associated with ASCVD in proportion to the degree of LDL-C changes (Fig. 1). Those facts could make us confident that LDL-C is one of the well-established causal factors for atherosclerotic cardiovascular disease (ASCVD) based on the findings from Mendelian randomization studies in addition to RCT. The beautiful consistency between RCT and Mendel randomization studies have reassured us that the lower, the better, as well as the earlier, the better appear to be true.

KEY WORDS: cholesterol, genetics, LDL, lipoproteins, PCSK9

II. Lessons from extreme cases

It is almost always useful to see the extreme cases to understand the relationship between particular biomarkers and outcomes. In the case of LDL-C and ASCVD, the most important as well as understandable situation could be familial hypercholesterolemia (FH) and ASCVD. For example, the patients with homozygous FH whose LDL-C levels typically elevated to as high as ~ 500 mg/dl exhibited premature ASCVD. Interestingly, several phenocopies of this situation with extremely high LDL-C, including autosomal recessive hypercholesterolemia (ARH) and sitosterolemia caused by different genetic mutations exhibit similar phenotypes, including, tendon/cutaneous xanthomas, and premature ASCVD, similar to those observed in homozygous FH (Fig. 2). Those cases simply indicate that LDL-C is the causal factor of this situation regardless of genetic etiology. On the other hand, findings from the patients exhibiting extremely low LDL-C with any genetic backgrounds could also tell us a lot about the relationship between LDL-C and ASCVD. Our patient with abetalipoproteinemia (ABL) caused by microsomal triglyceride transfer protein (MTTP) mutations (LDL-C = 0 mg/dl) did not exhibit any coronary plaque nor aortic calcifications at the age of 51 (Fig. 3), although he suffers from spinocerebellar ataxia, and retinal pigmentary degeneration due to lack of fat-soluble vitamin. On the contrary, we have shown an interesting case of homozygous familial hypobetalipoproteinemia (FHBL) whose LDL-C was as low as 1 mg/dl. He does not exhibit any complications relating fat-soluble vitamin deficiency.
described above, probably due to his preserved HDL cholesterol (HDL-C) level containing fat-soluble vitamin. It would be important to see none of the family members whose LDL-C was quite low had atherosclerotic diseases. Also, it would be quite interesting to understand that novel pharmacological interventions for LDL-lowering have been developed based on the findings obtained from those extreme cases (Table 1).

III. Lessons from Mendelian randomization studies

In any diseases, it should be critical, but difficult to think of “causal” factors. For example, Koch’s postulates are four criteria designed to establish a causative relationship between a microbe and a disease. It includes 1) The microorganism must be found in abundance in all organisms suffering from the disease, but...
should not be found in healthy organisms. 2) The microorganism must be isolated from a diseased organism and grown in pure culture. 3) The cultured microorganism should cause disease when introduced into a healthy organism. 4) The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent. How about in cardiovascular diseases? RCT should be the gold standard to establish a causal relationship between a particular biomarker and a disease. On the other hand, Mendelian randomization study is a technique that uses genotypes as instruments to assess a causal relationship between biomarkers and outcomes even in a cross-sectional manner^{17}. Using this scheme, genetic variants associated with LDL-C have been robustly associated with ASCVD, regardless of genes as

![Fig. 3](image_url) Images of coronary computed tomography and carotid ultrasound in a patient with ABL.
A: Coronary computed tomography obtained in a patient with ABL. There is no stenotic lesions nor any calcifications identified in coronary arteries. ABL: abetalipoproteinemia, RCA: right coronary artery, LAD: Left anterior descending coronary artery, LCX: Left circumflex coronary artery.
B: Carotid ultrasound image obtained in a patient with ABL. There is no stenotic lesions nor intima-media thickness in right common carotid artery.
C: Carotid ultrasound image obtained in a patient with ABL. There is no stenotic lesions nor intima-media thickness in left common carotid artery.

| Table 1 Novel pharmacological interventions for LDL-lowering |
|-------------------------------------------------------------|
| **Target** | Deficiency or carriers of PTV | **Compounds** | **Randomized controlled trials** | Mendelian randomization |
| NPC1L1 | Heterozygous carriers (1 in 650 individuals) | Ezetimibe | IMPROVE-IT (Ref 2) | Ref 7, 8 |
| PCSK9 | Familial hypobetalipoproteinemia | Evolocumab | FOURIER (Ref 3) | Ref 5, 6 |
| | | Alirocumab | ODYSSEY OUTCOMES (Ref 4) | |
| MTPP | Abetalipoproteinemia | Lomitapide | NA | Ref 6 |
| APOB | Familial hypobetalipoproteinemia | Mipomersen | NA | Ref 27 |
| ANGPTL3 | Familial combined hypolipoproteinemia | Evinacumab | NA | Ref 28 |
| ACLY | NA | Bempedoic Acid | NA | Ref 31 |

NPC1L1: Niemann-Pick C1-Like 1, PCSK9: proprotein convertase subtilisin-kexin type 9, MTPP: microsomal triglyceride transfer protein, APOB: apolipoprotein B, ANGPTL3: Angiopoietin-like 3, Ref: reference, NA: not available, ACLY: ATP citrate lyase.
stated above. It is interesting to note that genetic variants associated with HDL-C were not associated with ASCVD\(^{18,19}\), consistent with negative results of RCT targeting lower HDL-C\(^{20-22}\). When we add results obtained through RCT using statin, ezetimibe, PCSK9 inhibitors, and a cholesteryl ester transfer protein (CETP) inhibitor, we could assume that earlier intervention for lowering LDL-C might be much better (the earlier, the better), since the slope of the regression line from genetic studies is steeper than that from RCT, in addition to the fact that the lower, the better (Fig. 1). In addition, we can estimate that “LDL-C” is the causal factor for ASCVD because of the linear relationship between LDL-C and ASCVD regardless of genes or drugs (Fig. 1). Accordingly, so-called “pleiotropic” effect of LDL-C lowering drugs appear to be minimal from those observations. Moreover, Table 2 summarizing the results obtained through RCT and Mendelian randomization studies focusing on protein truncating variants (extreme situations) clearly indicates that super aggressive as well as earlier LDL-C lowering should be beneficial.

### IV. Lessons from recent RCT with super aggressive LDL-C lowering therapies

Since the establishment of clinical usefulness of statins, there are debates regarding super aggressive LDL-C lowering therapies, including targeting much lower than 100 mg/dl, as well as the additional drugs on top of statins. Regarding the first matter, a RCT named EMPATHY study, targeting LDL-C level ≤ 70 mg/dl using mainly statins among high-risk Japanese diabetic patients with primary prevention setting revealed beneficial effect\(^{23}\). In this study, patients receiving aggressive LDL-C lowering therapies (mean LDL-C level was 76.5 mg/dl) exhibited significantly lower ischemic stroke events than those with standard care (mean LDL-C level was 104.1 mg/dl). Moreover, high-dose statin therapy reaching to LDL-C level at 76.6 mg/dl has been shown to be better than low-dose statin therapy reaching to LDL-C level at 91 mg/dl among Japanese secondary prevention patients\(^{24}\). As for the second matter, recent mega RCT using ezetimibe, PCSK9 inhibitors, and a CETP inhibitor on top of statins consistently revealed that additional beneficial effects could be obtained through such super-aggressive LDL-C lowering therapies in proportion to the absolute degree of LDL-C lowering\(^{25,26}\). It is of note that ASCVD events seemed to decline with achieved LDL-C, to a level of approximately 30 mg/dl in ODYSSEY OUTCOMES (using alirocumab)\(^{25}\), and to a level of approximately 10 mg/dl in FOURIER (using evolocumab)\(^{27}\). Those observations collectively make us confident that the lower, the better could be applicable, at least at the range of LDL-C ~ 30 mg/dl.

### V. Lessons from professors Brown and Goldstein

In addition to the observations from those RCT, professors Brown and Goldstein, both of whom are Nobel laureates, suggested that the levels of cholesterol in our industrialized societies are inappropriately high\(^{28}\). This comment was derived from 3 different important aspects of nature, 1) a level of LDL-C in plasma of 25 mg/dl would be sufficient to nourish body cells with cholesterol, estimated by the experimental studies showing that LDL receptor binds LDL optimally when the lipoprotein is present at a cholesterol concentration of 2.5 mg/dl. And it has been shown that there is a 10-to-1 gradient between concentrations of LDL in plasma and interstitial fluid, 2) plasma LDL-C levels of other mammalian without development of atherosclerosis are generally less than 80 mg/dl, 3) LDL-C level in new born humans is approximately 30 mg/dl, 4) when humans are raised on a low fat diet, the plasma LDL-C levels tend to stay in the range of 50 to 80 mg/dl.

### VI. Lessons from monkeys, our estimable ancestors

Let me remind you that LDL-C levels of monkeys, who are our estimable ancestors, have been shown as low as ~ 30 mg/dl\(^{29}\). Typically, wild monkeys have to survive in a natural field, requiring LDL-C because of the incident of bleedings and/or infections. Accordingly, it could be skeptical that humans, especially, those living in an industrialized societies need LDL-C level as high as ~ 100 mg/dl. In this regard, “standard” levels are

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**Table 2** Effects of randomized controlled trials and Mendelian randomization study in PTV on LDL-C and on ASCVD

| Gene     | RCT                        | Mendelian randomization study in PTV |
|----------|---------------------------|-------------------------------------|
|          | Trial name | LDL-C reduction (mg/dl) | ASCVD reduction (%) | LDL-C reduction (mg/dl) | ASCVD reduction (%) |
| APOB     | NA         | NA                     | NA                    | 43                    | 72                    |
| CETP     | REVEAL     | 26                     | 9                     | 12                    | 30                    |
| NPC1L1   | IMPROVE-IT | 17                     | 6                     | 12                    | 53                    |
| PCSK9    | FOURIER/ODYSSEY | 62/48               | 15/15                  | 21                    | 88                    |

RCT: randomized controlled trial. PTV: protein truncating variant. ASCVD: atherosclerotic cardiovascular disease. APOB: apolipoprotein B. CETP: cholesteryl ester transfer protein, NPC1L1: Niemann-Pick C1-Like 1, PCSK9: proprotein convertase subtilisin-kexin type 9, NA: not available.
usually determined based on “average” values, not bade on “healthy” values in any biomarkers, including LDL-C. Thus, it would be better to re-think of “standard” levels of cholesterol.

VII. Future world where Mendelian randomization studies precede RCT

As stated above, Mendelian randomization studies could be quite useful to predict usefulness as well as disadvantages of potential medical therapies. By now, Mendelian randomization studies investigating LDL-C matters have just confirmed the positive causal association between LDL-C and ASCVD only after the publish of RCT. However, several investigations, including ours have already preceded the results from RCT in the cases of apolipoprotein B (APOB) as well as angiotenisin-like 3 (ANGPTL3). We have shown that the individuals with APOB protein truncating variants exhibited significantly reduced risk of ASCVD\(^3\). Also, similar results were obtained in the case of ANGPTL3\(^3\).\(^5\). Moreover, a Mendelian randomization study focusing on ATP citrate lyase (ACLY), which is an enzyme of cholesterol–biosynthesis pathway located upstream of 3-hydroxy-3-methylglutaryl–coenzyme A reductase (HMGCR)\(^3\). It is quite interesting to note that the beneficial effect of ATP citrate lyase inhibitor on ASCVD prevention have been shown just after the issue of clinical phase 3 trial of ATP citrate lyase inhibitor\(^3\). The clinical usefulness will be shown by the RCT in both cases; however, we are quite confident that those issues have now been well predicted through Mendelian randomization studies.

VIII. Another beneficial aspect of super aggressive LDL-C lowering therapies

There are several causal risk factors other than LDL-C for the development of ASCVD, including hypertension, and non-fasting blood glucose. However, we know that a simple the lower, the better story may not be applicable to those risk factors, especially, in the case with non-fasting blood glucose where aggressive blood glucose lowering therapies have been associated with worse outcomes\(^3\).\(^5\).\(^6\).\(^8\).\(^9\).\(^11\).\(^12\) We usually do not have hesitations to use multiple drugs to reduce blood pressure, and blood glucose, although those could lead to worse outcomes. On the other hand, it is not quite popular to do so in an LDL-C lowering therapy despite a plenty of evidence revealing that the lower, the better.

IX. Conclusion

In this paper, we have repeatedly emphasized that LDL-C is a causal risk factor for ASCVD. Also we have learned from lines of evidence that super-aggressive LDL-C lowering therapies, at least around 30 mg/dl could be safe. Much more attention should be given to LDL-C for additional reduction of ASCVD events.

Highlights

- LDL-C is a causal risk factor for ASCVD.
- Super-aggressive LDL-C lowering therapies, at least around 30 mg/dl could be safe.
- Genetic studies could be useful to determine true causal factors for human diseases.

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Declarations of interest

None declared.

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