



Clinical Perspectives of Genetic Analyses on Dyslipidemia and Coronary Artery Disease

Hayato Tada, Masa-aki Kawashiri and Masakazu Yamagishi

Department of Cardiovascular and Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

We have learned that low-density lipoprotein (LDL) cholesterol is the cause of atherosclerosis from various aspects, including a single case with familial hypercholesterolemia, other cases with different types of Mendelian dyslipidemias, large-scale randomized controlled trials using LDL cholesterol lowering therapies, and Mendelian randomization studies using common as well as rare variants associated with LDL cholesterol levels. There is no doubt that determinations of genotypes in lipid-associated genes have contributed not only to the genetic diagnosis for Mendelian dyslipidemias but also to the discoveries of novel therapeutic targets. Furthermore, recent studies have shown that such genetic information could provide useful clues for the risk prediction as well as risk stratification in general and in particular population. We provide the current understanding of genetic analyses relating to plasma lipids and coronary artery disease.

Key words: Dyslipidemia, Familial hypercholesterolemia, PCSK9, Coronary artery disease

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Introduction

Familial hypercholesterolemia (FH; OMIM #143890) is characterized by the triad of (1) primary hyper-LDL-cholesterolemia, (2) tendon xanthomas, and (3) premature coronary artery disease (CAD)^{1, 2}. Since the identification of LDL receptor in 1970s, as the cause of FH, three different genes have been shown to cause this clinical phenotype. Genetic analyses for the patients with FH have provided not only definitive diagnosis but also the chances of cascade screening for their offsprings³. Moreover, investigations for the genetic background for FH led to the development of novel therapeutic targets, such as proprotein convertase subtilisin/kexin type 9 (*PCSK9*) and apolipoprotein B (*ApoB*). Moreover, observations on other Mendelian dyslipidemias exhibiting LDL cholesterol elevation, such as sitosterolemia, and autosomal recessive hypercholesterolemia (ARH) convince us the causal role of LDL cholesterol on atherosclerosis⁴⁻⁶. Recent advances of comprehensive genetic analyses made us

realize the genetic architecture comprising rare variants with large effect sizes causing Mendelian dyslipidemia and common variants with small effect sizes causing polygenic dyslipidemias⁷. Due to such advances, we are now understanding that determination of rare as well as common genetic variants associated with lipids and CAD are quite useful not only in research settings but also in clinical settings.

Lessons from Patients with FH and Other Mendelian Dyslipidemias

(1) LDL Cholesterol as a Causal Factor for the Development of CAD

FH is one of the most common monogenic Mendelian inherited disorders characterized by excess deposition of cholesterol in tissues leading to tendon xanthomas and premature CAD^{1, 2}. It has been well known that FH is caused by genetic mutations of LDL receptor or its related proteins, including *ApoB*, or *PCSK9*, although a fraction of patients with clinical FH were unexplained by mutations in those genes^{1, 2}. It is of note that even a single encounter with a patient with FH harboring an LDL receptor mutation could tell us that LDL cholesterol is a causal factor of CAD. Moreover, the fact that individuals with different mutation(s) in different gene(s) that significantly elevated LDL cholesterol level exhibit premature CAD

Address for correspondence: Hayato Tada, Department of Cardiovascular and Internal Medicine, Kanazawa University Graduate School of Medicine, 13-1 Takara-machi, Kanazawa, 920-8641, Japan
E-mail: ht240z@sa3.so-net.ne.jp
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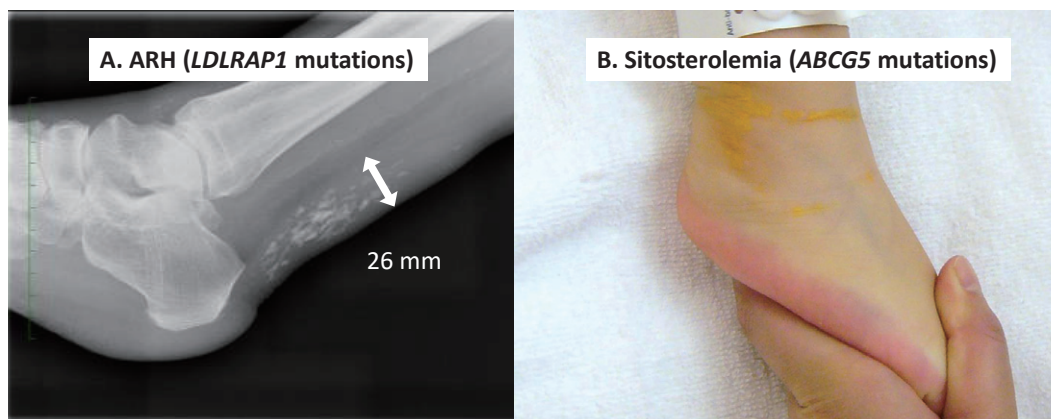


Fig. 1. Mendelian dyslipidemias exhibiting hyper LDL cholesterolemia. A. Autosomal recessive hypercholesterolemia caused by LDL receptor adaptor protein 1 gene mutations. B. Sitosterolemia caused by ATP-binding cassette subfamily G member 5 gene mutations.

strongly convince us that LDL cholesterol is the causal factor of CAD, including ARH caused by LDL receptor adaptor protein 1 (*LDLRAP1*) gene mutations and sitosterolemia caused by ATP-binding cassette subfamily G member 5/8 (*ABCG5/ABCG8*) gene mutations (**Fig. 1**). Investigations on such extreme cases exhibiting Mendelian form of inheritance caused by (a) striking mutation(s) are one of the best ways to understand the roles of particular molecules and the causal relationship between a biomarker and an outcome.

(2) Clinical Impact of Genetic Analyses for FH-Associated Gene

Currently, genetic analyses for FH-associated genes are mostly conducted in research settings in most of the country, including Japan, since its cost cannot be covered by health insurance. Moreover, such analyses have been suggested as a pure diagnostic tool when the clinical diagnosis of FH is unclear by most of the guidelines⁸⁻¹⁰. Based on such backgrounds, few data had existed regarding the clinical impact of determination of genetic status of FH in the patients with elevated LDL cholesterol level, which could aid us for risk stratification in the patients suspected as FH. In this regard, we have assessed the prevalence of an FH mutation among those with severe hypercholesterolemia and investigated whether CAD risk varies according to mutation status beyond the observed LDL cholesterol level and found that FH mutation status had substantially increased risk for CAD (**Fig. 2**)¹¹. Given that genetic analyses for FH-associated genes needed to be assessed only once in a lifetime could help identifying the individuals with the highest risk far before the development of other traditional acquired risk factors, such as hypertension and

diabetes among the patients with elevated LDL cholesterol level, it may be reasonable that such costs be covered by insurance.

(3) Clinical Application of whole Exome Sequencing for Mendelian Dyslipidemias

Usefulness of whole exome sequencing (WES) has been shown in many Mendelian inherited diseases, especially, in cases with recessive form of inheritance^{12,13}. We have demonstrated that WES is feasible even where DNA is available in only one individual if the recessive form of inheritance is firmly established in a family with Tangier disease caused by ATP-binding cassette transporter A1 gene mutations¹². Moreover, WES could identify causative mutations in different genes simultaneously, where patients suffered from different Mendelian inherited diseases, such as sitosterolemia and familial Mediterranean fever⁵. Furthermore, we also identified a rare case with heterozygous FH caused by de novo LDL receptor mutation exhibiting recessive form of inheritance using WES¹⁴.

(4) Challenges of Identifying of Novel FH Genes

We could not determine causative mutations within established three FH genes (*LDL receptor*, *ApoB*, and *PCSK9*) in up to 40% of the cases with clinically diagnosed as FH. Such a fact has motivated researchers to identify novel genes causing this situation among the patients with genetically undetermined FH. We conducted WES on 213 selected family members from 41 kindreds with suspected Mendelian inheritance of extreme levels of LDL cholesterol or high-density lipoprotein (HDL) cholesterol (mainly LDL cholesterol); however, we failed to find any novel genes related with this disease (**Fig. 3**)¹⁵. Several potential reasons could be considered. First, it is still hard to determine struc-

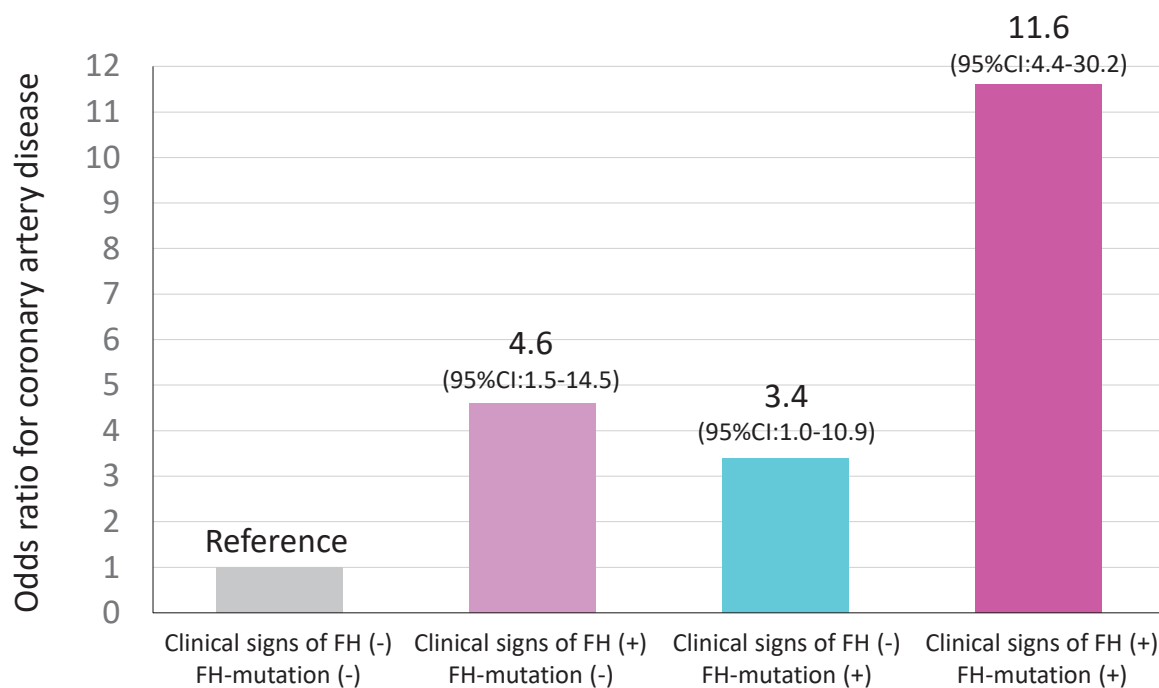


Fig. 2. Impact of clinical signs and genetic diagnosis of familial hypercholesterolemia on risk of coronary artery disease. Odds ratios were calculated using logistic regression after adjustment for age, sex, hypertension, diabetes, smoking, and LDL cholesterol levels.

tural variations by WES. Accordingly, further improvements in the application of approaches to discover structural variations using WES are needed. Secondary, large numbers of shared rare alleles within families obfuscated causal variant identification. We still need a large pedigree to identify a causal gene in a dominant mode of inheritance. Third, we found that ~15% of the families suspected as Mendelian dyslipidemia could be polygenic carrying a significant burden of common lipid-related alleles, rather than one or two critical mutations. It is still hard to define polygenic Mendelian dyslipidemias, since not all variations contributed to this phenotype have not been discovered.

(5) Polygenic FH

In 2013, Humphries and colleagues have demonstrated that the phenotype as FH could be caused by the excess of LDL cholesterol-raising common variants in a substantial proportion of patients with FH without a known mutation, in contrast to a situation where a single rare variant causes significant elevation in LDL cholesterol level (**Fig. 4**). Similar results were obtained in our analyses mentioned earlier, and we estimated up to 15% of the families suspected as Mendelian dyslipidemia could be polygenic cause¹⁶.

Heritability from Common and Rare Variants

Plasma lipids and lipoproteins are heritable risk factors for CAD, with heritability estimates ranging from 40% to 60%. Genome-wide association studies (GWASs) for plasma lipids and CAD have successfully identified 157 gene regions for plasma lipids¹⁷ and 58 for CAD¹⁸. Despite the success of GWAS, single-nucleotide polymorphisms (SNPs) at these loci explain only a modest proportion of the heritability—20%–25% of the heritability for plasma lipids and <10% of the heritability of CAD. These observations have led to the general question of how to account for the unexplained heritability. To estimate such contributions, several rare variant association studies investigating on rare variants and lipids using whole genome sequencing and imputation-based approach have been performed. Those investigations have found that rare and low-frequency variants account for 3%–4% of the variance explained in lipids¹⁹⁻²¹. In contrast, we have demonstrated that fair portion of the missing heritability for lipid traits can be explained by multiple common variants at each known locus²².

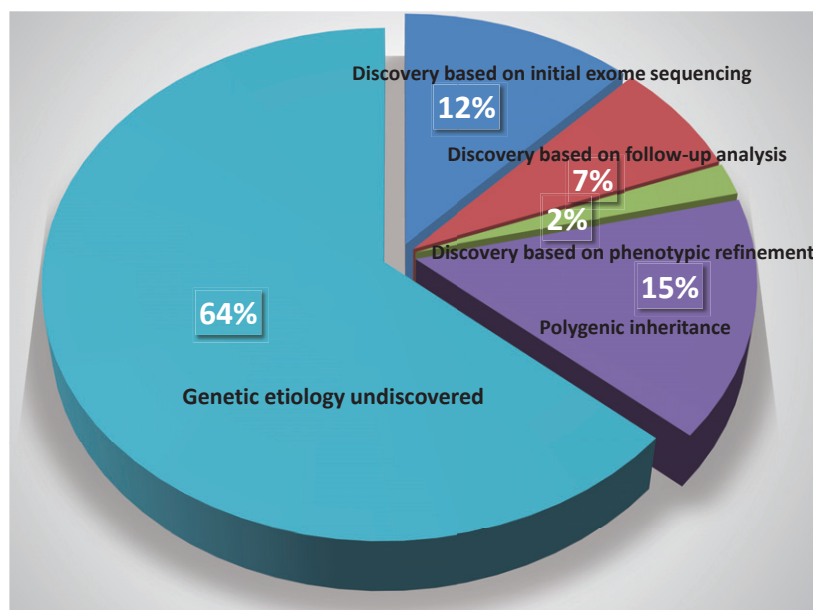


Fig. 3. Yield of whole exome sequencing for Mendelian dyslipidemias. The graph shows the percentage of final status using whole exome sequencing of the 41 families suspected as Mendelian dyslipidemias.

Genetic Risk Score for Risk Prediction of CAD

It is well known that CAD is highly heritable motivating the inclusion of family history of CAD as an option for risk assessment in patients with borderline risk. Moreover, researchers have tried to identify genetic clues associated with CAD and found as many as 58 independent loci associated with CAD. However, any guideline so far does not recommend the use of any genetic marker for the risk assessment of CAD due to lack of evidence²³. In this regard, Kathiresan and colleagues initially organized an interesting study investigating if aggregating information of individual SNPs (at that time 13 SNPs) could increase power of risk prediction in 2010²⁴. After that, updated genetic risk scores (GRSs) using increasing number of SNPs showed substantial clinical usefulness beyond traditional risk factors, including family history (**Table 1**). It is of note that such GRS could improve risk prediction far beyond family history information²⁵. Moreover, we can assess GRS quantitatively far before the development of traditional risk factors²⁶. Interestingly, a recent study showed that a favorable lifestyle was associated with a nearly 50% lower relative risk of CAD than was an unfavorable lifestyle among individuals at high GRS²⁷. Another study showed that individuals with the highest GRS derived the largest relative and absolute clinical benefit from statin therapy²⁸. Those facts collectively suggested that genetic

predisposition to CAD is not deterministic, or rather, genetic risk might be attenuated by a favorable lifestyle and/or statin therapy. Accordingly, it may be a prime time for us to consider GRS as a risk assessment tool in clinical settings.

Mendelian Randomization (Common and Rare)

To establish a causal relationship between a risk factor (usually a biomarker) and an outcome, randomized controlled trials (RCTs), which require a large amount of time and effort, are the gold standard. In contrast, the Mendelian randomization study is a technique that uses genotypes as instruments to assess a causal relationship between biomarkers and outcomes. In a Mendelian randomization study, a genetic variant associated with a particular biomarker is used as a proxy for the biomarker. Outcomes are compared between the group harboring the effect allele and a group with the reference allele. This approach can be considered a proxy for an RCT, in which the randomized groups have similar confounding variables. Accordingly, a Mendelian randomization study can be regarded as a natural RCT.

(1) Effect on Cardiovascular Diseases

A number of biomarkers have been shown to be associated with CAD from epidemiological studies. However, careful attention should be paid for the fact

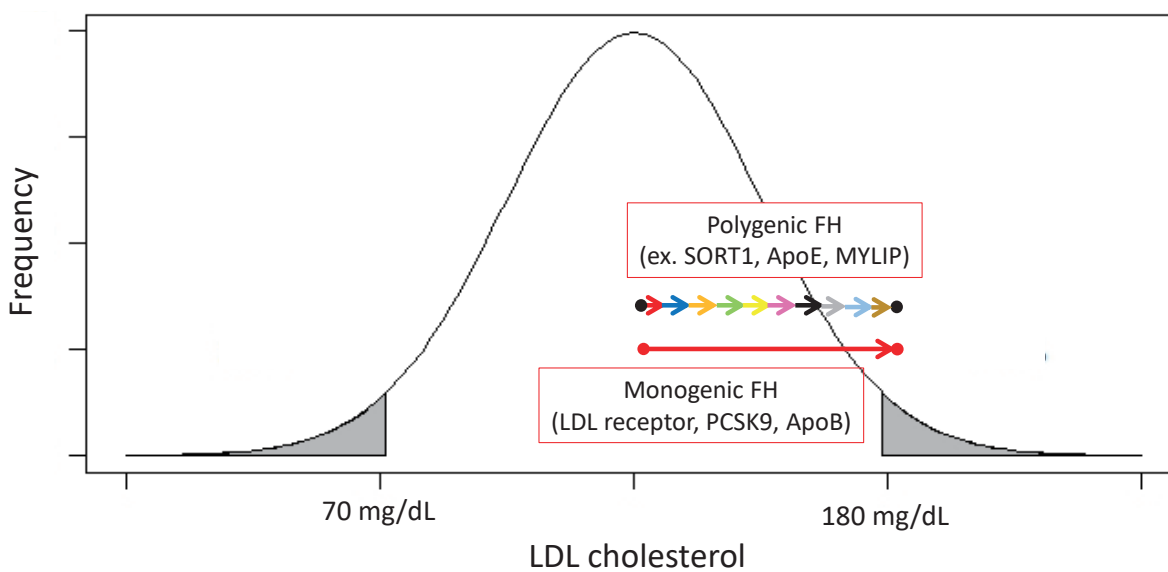


Fig. 4. Monogenic familial hypercholesterolemia (FH) and polygenic FH. X-axis represents LDL cholesterol value. Y-axis represents frequency. A single rare variant with large effect size or multiple single-nucleotide polymorphisms with modest effect sizes could elevate LDL cholesterol level substantially.

Table 1. Comparison of power between traditional risk factors and GRS

Baseline characteristics	HR (95% CI)	P value
Age (per 10-year increment)	1.86 (1.74 - 1.98)	2.8×10^{-83}
Men	2.50 (2.28 - 2.74)	1.4×10^{-84}
Body mass index (top vs. bottom quintile)	1.07 (0.92 - 1.24)	0.38
Smoking	1.81 (1.66 - 1.98)	1.1×10^{-38}
Hypertension	1.69 (1.53 - 1.86)	1.6×10^{-25}
Prevalent diabetes mellitus	2.43 (2.12 - 2.78)	4.0×10^{-37}
Apolipoprotein B (top vs. bottom quintile)	2.39 (2.03 - 2.82)	9.0×10^{-26}
Apolipoprotein A1 (bottom vs. top quintile)	1.87 (1.61 - 2.18)	5.0×10^{-16}
Self-reported family history	1.41 (1.29 - 1.53)	2.9×10^{-15}
GRS (top vs. bottom quintile)	1.85 (1.61 - 2.12)	1.2×10^{-18}

HR: hazard ratio, GRS: genetic risk score

that those biomarkers are causal or not. In the case with LDL cholesterol, many epidemiological studies, as well as Mendelian randomization studies, using rare as well as common genetic variants consistently showed that the directions and magnitude by LDL cholesterol level and the odds for CAD were linearly correlated. Moreover, those odds induced by genetically determined LDL cholesterol levels were consistently larger than those induced by pharmacological interventions (**Fig. 5**). In contrast, common genetic variants in HDL-raising genes did not reduce the risk of CAD^{29, 30}. Interestingly, plasma triglyceride and lipoprotein (a) (Lp(a)) have been shown to be associated with CAD in Mendelian randomization studies, suggesting their

causal roles for the development of CAD³¹.

Regarding other variables, a large-scale Mendelian randomization study has investigated the causal role of uric acid on CAD, heart failure, ischemic stroke, type 2 diabetes, and gout. They found that serum uric acid levels, increased by the common genetic backgrounds, were not associated with CAD, heart failure, ischemic stroke, or type 2 diabetes³². Moreover, the patients with Lesch–Nyhan syndrome exhibiting increased levels of uric acid caused by rare mutations in hypoxanthine phosphoribosyltransferase 1 gene manifests with severe hyperuricemia since birth, gout, renal stones, and neurological impairment but not CAD, heart failure, ischemic stroke, or type 2 diabe-

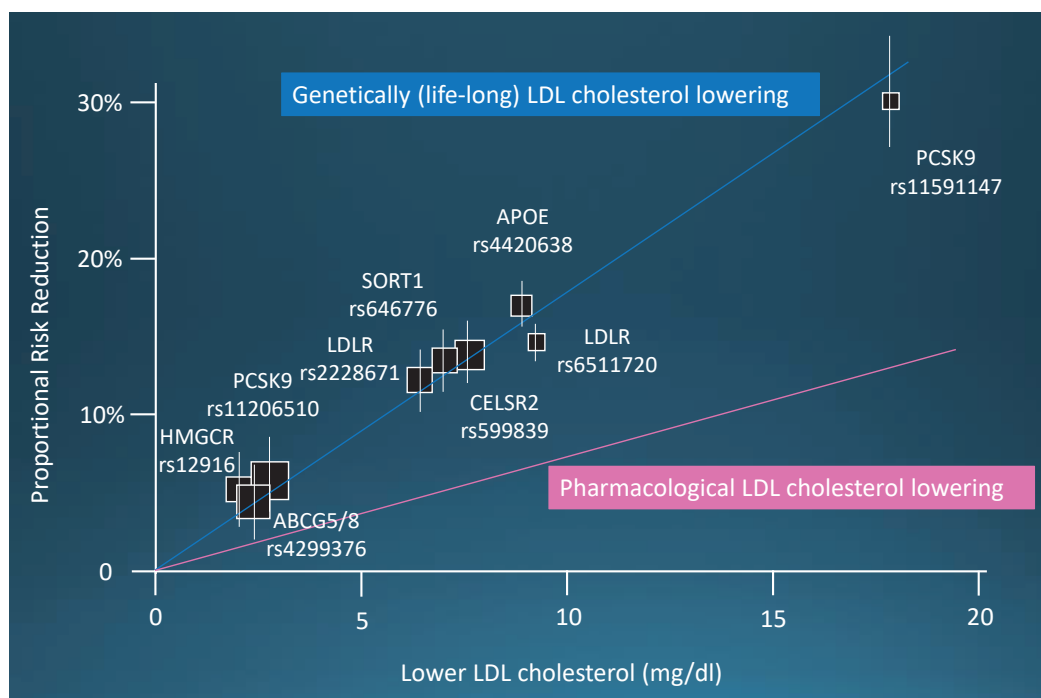


Fig. 5. Relationship between coronary artery disease (CAD) risk reduction and LDL cholesterol lowering. Blue line indicates the relationship between CAD risk reduction and genetic (lifelong) LDL cholesterol lowering. Pink line indicates the relationship between CAD risk reduction and pharmacological lowering of LDL cholesterol.

tes. Those findings from common as well as rare variants suggest that uric acid does not seem to be causally associated with cardiovascular diseases³³.

(2) Effect on Diabetes

A meta-analysis of RCT using statin showed consistent effectiveness of LDL cholesterol lowering therapy on reduction of CAD. However, it also revealed that LDL cholesterol lowering therapy using statin increased the risk of diabetes³⁴. Soon after this report, a Mendelian randomization study focusing on the effects of LDL cholesterol lowering and development of diabetes by variants in HMG-coenzyme A reductase (*HMGCR*) gene, which is the target of statin. They found that loss of function variants in *HMGCR* gene is associated with reduced LDL cholesterol level as well as increased risk of diabetes, which is consistent with RCT³⁵. Next critical questions are (1) how about other therapies, such as ezetimibe, *ApoB*, and *PCSK9* and (2) how about rare variants with large effect size, such as the patients with FH. For the first question, a meta-analysis focusing on this point revealed that LDL cholesterol lowering alleles were associated with reduced risk of CAD as well as increased risk of diabetes regardless of gene. Such a notion could lead us to speculate that LDL cholesterol is inversely but weakly

associated with diabetes³⁶. For the second question, a large-scale, simple observational study has suggested that the prevalence of diabetes in the patients with FH is smaller than that of non-FH, suggesting that exposure of high LDL cholesterol level could be protective from diabetes³⁷. Similarly, we can estimate any side effects by novel drugs inhibiting a particular molecule by investigating Mendelian diseases with large effect size or Mendelian randomization studies using common variants in the gene of interest.

Conclusion

It is well known that dyslipidemias and CAD are highly heritable diseases, motivating us to investigate their genetic backgrounds for a long time. Now, we have learned that common as well as rare genetic variants are contributing to such “heritability.” Systematic genetic analyses should be able to provide more clinically relevant information for the assessments of the patients with dyslipidemias and CAD in the near future.

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Conflicts of Interest Disclosures

None.

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