

아주대학교 NGR

Mendelian Randomization and Causality in Stroke Research



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박 광 열

OPEN

Role of Blood Lipids in the Development of Ischemic Stroke and its Subtypes

A Mendelian Randomization Study

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Matthew Traylor, PhD; Hugh S. Markus, DM; Olle Melander, MD, PhD;
Marju Orho-Melander, PhD; on behalf of the Stroke Genetics Network (SiGN)

JAMA | Original Investigation

Association of Triglyceride-Lowering *LPL* Variants and LDL-C-Lowering *LDLR* Variants With Risk of Coronary Heart Disease

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Hindy G et al Stroke 2018; Ference BA et al JAMA 2019

Concept of MR

- 정의: 유전형(G)이 반영하는 위험요인(X)의 건강영향(Y)을 유전적 도구의 무작위배정효과를 통해 인과적 관련성으로 평가하며, $G \rightarrow X$, $G \rightarrow Y$ 의 종합분석을 통해서 X의 변화가 Y에 미치는 정량적인 위험도의 크기를 평가한다.
- 연구대상자를 유전형별로 나누면, 해당 유전자좌의 유전형 이외에는 두 군 간에 차이가 없음 (like RCT).

Objective

Mendelian randomization is
the use of **genetic variants**
as **instrumental variables (IVs)**
in non-experimental design
to make **causality** of a modifiable exposure on an outcome or disease.

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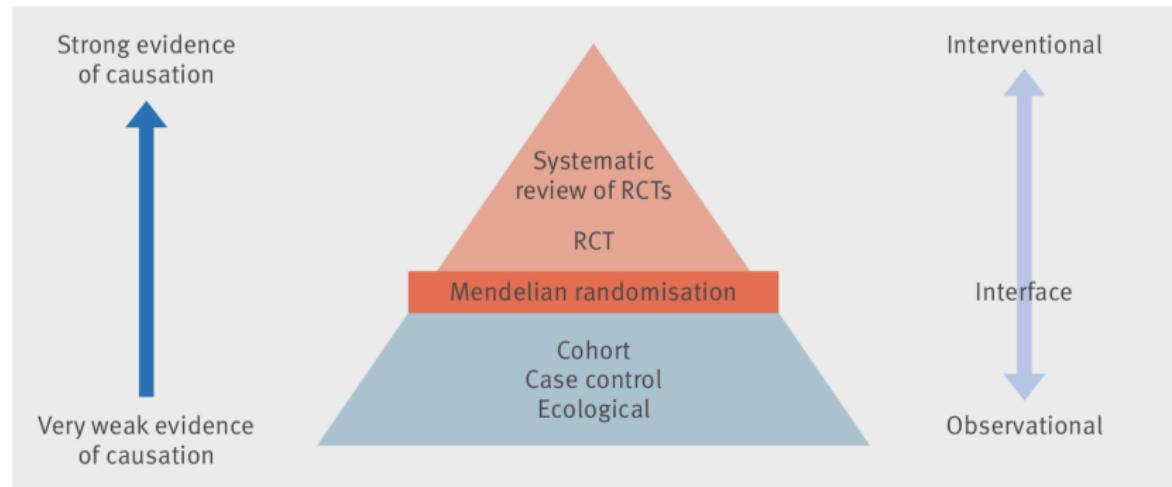
④ MR study in Stroke and CHD

⑤ Summary

Why MR is useful?

Mendelian randomization is
the use of **genetic variants**
as **instrumental variables (IVs)**
in **non-experimental design**
to make **causality** of a modifiable exposure on an outcome or disease.

Observational study and RCT



Strengths and Limitations

Observational study

- ① Easy to perform
- ② Errors in measurement
 - Variability
 - Exposure in the past
- ③ Confounders
 - Unmeasured
- ④ Reverse causation

RCT

- ① Easy to control confounders
- ② Expensive
- ③ Ethical problems
- ④ Short observation period
- ⑤ Limited applicability to real-world

Solutions

	Observational study	RCT	MR
Measurement error	Reliable measuring	Reliable measuring	Life-long effects
	Biomarker 활용	Biomarker 활용	
Confounders	Consideration of confounders	Randomization	Randomization
Causation	Biological explanation	Possible	Possible

Genetic variant ?

Mendelian randomization is

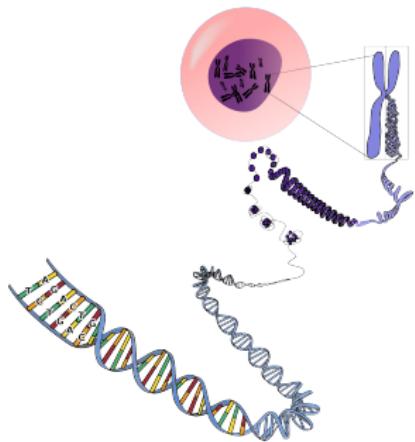
the use of **genetic variants**

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to make **causality** of a modifiable exposure on an outcome or disease.

Size of DNA variation



- Whole chromosomal and whole genome changes: ~1-6 billion bp
 - Aneuploidy (abnormal number of chromosomes)
 - Aneusomy (fewer or more copies than 2 of a chromosome)
 - Interchromosomal translocations
 - Ring chromosomes
 - Microscopic to submicroscopic
 - Segmental aneusomy
 - Chromosomal deletions
 - Chromosomal insertions
 - Chromosomal inversions
 - Intrachromosomal translocations
 - Chromosomal abnormality
 - Fragile sites
 - 1 kb to submicroscopic
 - Copy number variants (CNVs)
 - Segmental duplications
 - Inversions, translocations
 - CNV regions
 - Microdeletions
 - Microduplications
 - 2 bp to 1,000 bp
 - Microsatellites, minisatellites
 - Insertion-deletions (Indels)
 - Inversions
 - Di-, tri-, tetra-nucleotide repeats
 - Variable number tandem repeats e.g. microsatellites
 - Single nucleotide 1 bp
 - Indels
 - SNPs
- bp indicates base pairs; SNP single nucleotide polymorphisms.

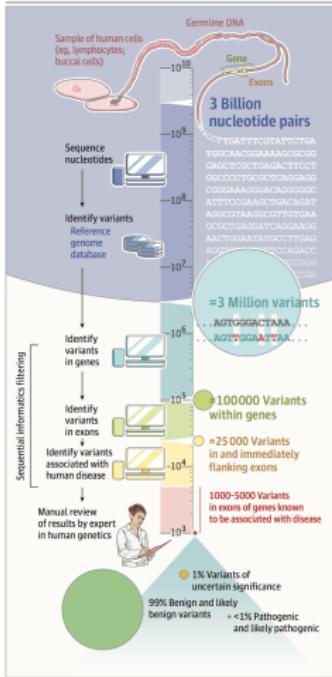
Cytogenetic
changes



Molecular
genetic
changes

- Interpretation of information
 - 3,000,000,000 base pairs
 - 3,000,000 SNPs
 - 100,000 variants in exon
- significance level in GWAS:
 $5 * 10^{-8} = 0.05 * 10^{-6}$
- rs******(number):
 Reference SNP cluster ID

Figure. Informatic and Human Analysis Required for Finding Rare Pathogenic Variants in a Human Genome



Instrument variable ?

Mendelian randomization is

the use of **genetic variants**

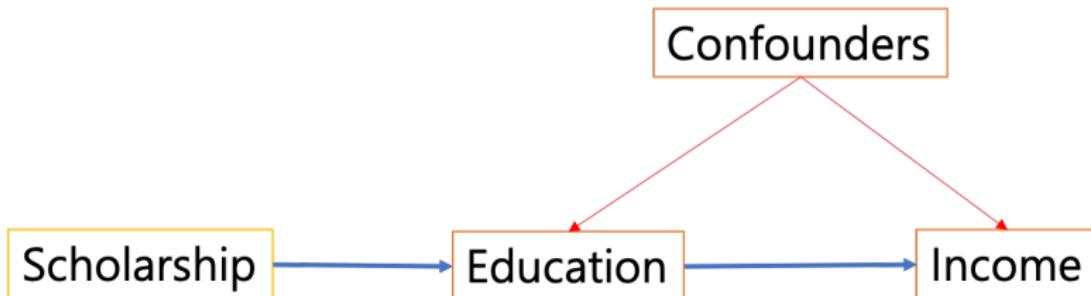
as **instrumental variables (IVs)**

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Instrumental variable

- Question: May education lead to higher income?
- IV: Scholarship (random allocation)



Scholarship and wealth of the family

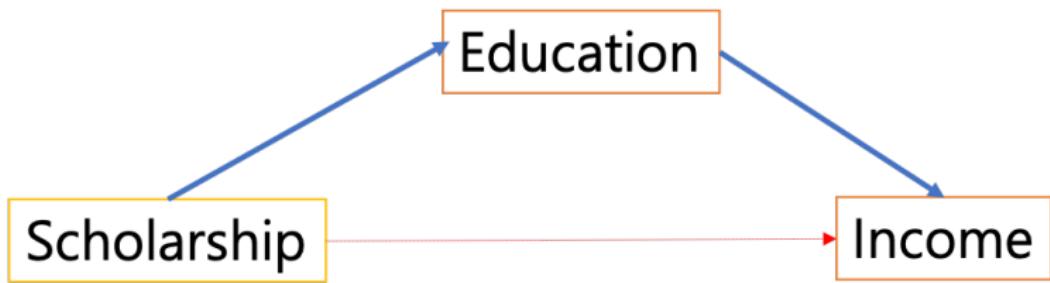
Scholarship +

- Rich family
less effect on education
- Poor family
promote education

Scholarship -

- Rich family
less effect on education
- Poor family
does not promote
education

Instrumental variable



Genetic variants as Instrument variable ?

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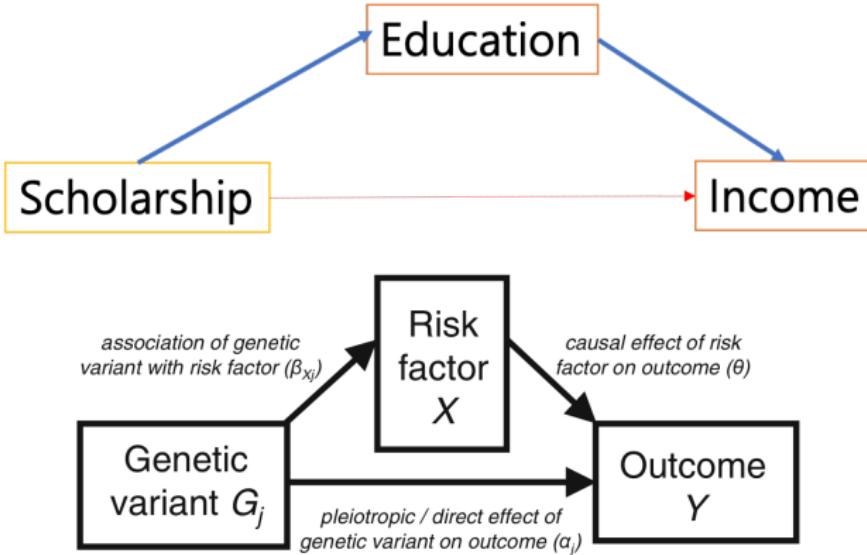


Fig. 1 Decomposing association for genetic variant G_j with the outcome into an indirect (causal) effect via the risk factor and a direct (pleiotropic) effect (see Eq. 1)

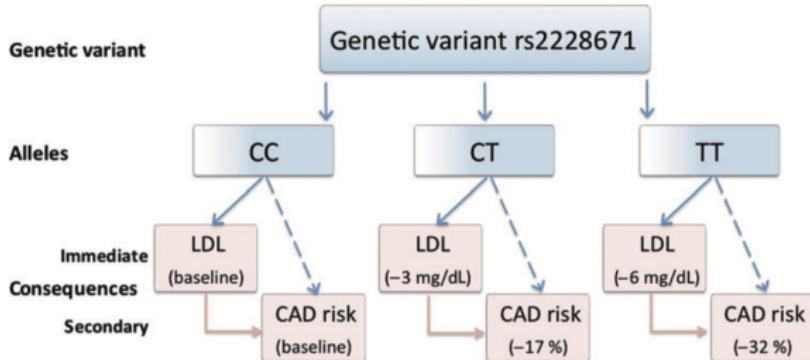


Figure 2 The effects of rs2228671 genotypes in the LDL receptor gene on LDL cholesterol (mg/dL) and coronary artery disease risk (% risk change) are shown as assessed by Linsel-Nitschke *et al.* across different cohorts comprising data from about 9000 individuals.¹⁶ The decrease of LDL serum concentration and the decrease in coronary artery disease risk go in parallel the number of T alleles. Since the gene has no other known functions it can be assumed that the LDL increase is causally involved in coronary artery disease.

What is the law of independent assortment?

Mendel's law of independent assortment states that the alleles of two (or more) different genes get sorted into gametes independently of one another. In other words, the allele a gamete receives for one gene does not influence the allele received for another gene.

서로 다른 두 유전자는 독립적으로 유전이 된다.

<https://www.khanacademy.org/science/high-school-biology/hs-classical-genetics/hs-introduction-to-heredity/a/the-law-of-independent-assortment>

What is the law of independent assortment?

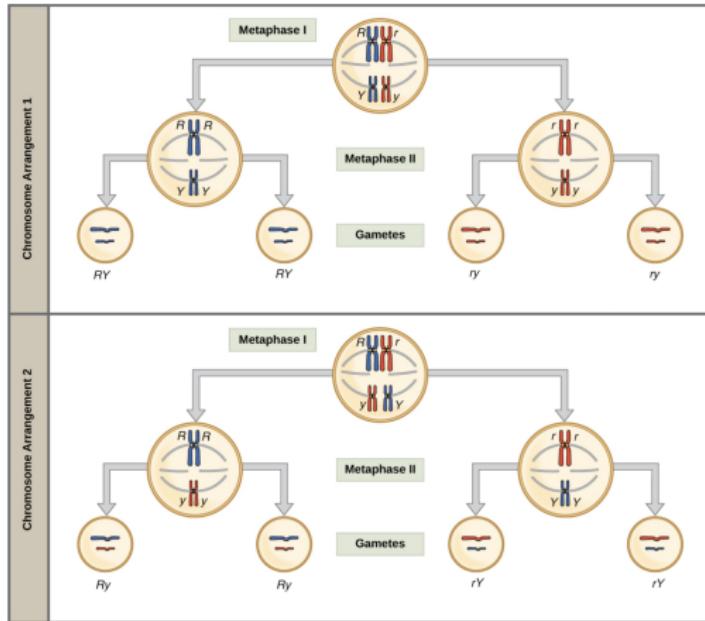


Image modified from "The laws of inheritance: Figure 5" by OpenStax College, Concepts of Biology, CC BY 4.0

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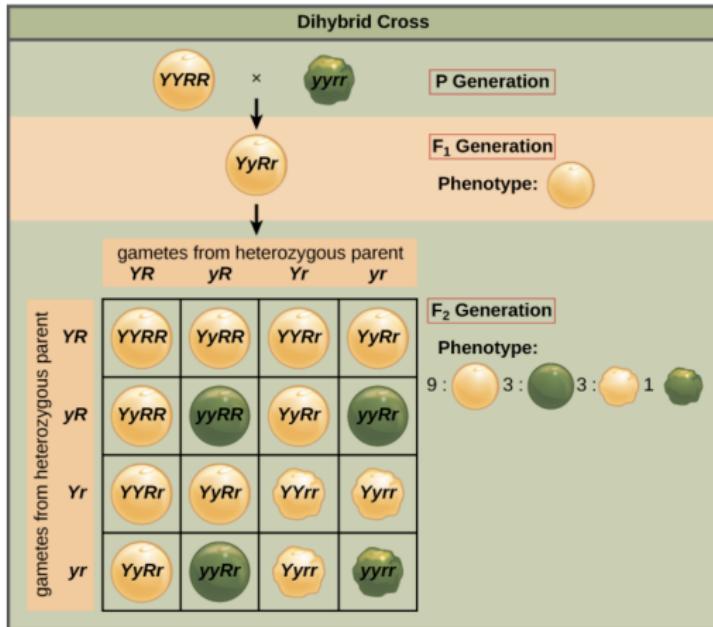
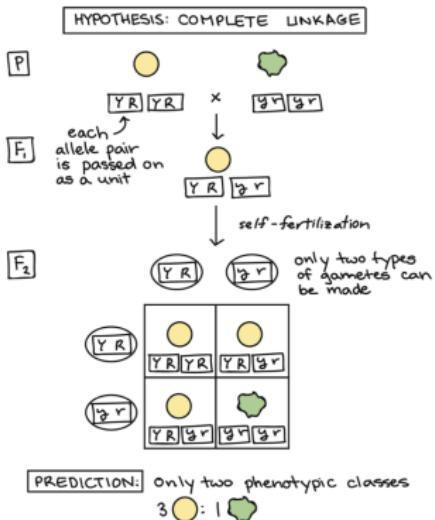


Image credit: "Laws of inheritance: Figure 2," by OpenStax College, Biology, CC BY 4.0.

<https://www.khanacademy.org/science/high-school-biology/hs-classical-genetics/hs-introduction-to-heredity/a/the-law-of-independent-assortment>

Linkage



Rather than giving a color allele and, separately, giving a shape allele to each gamete, the F₁ dihybrid plant would simply give one "combo unit" to each gamete: a YR allele pair or a yr allele pair.

<https://www.khanacademy.org/science/high-school-biology/hs-classical-genetics/hs-introduction-to-heredity/a/the-law-of-independent-assortment>

Genetic variants as Instrument variable ?

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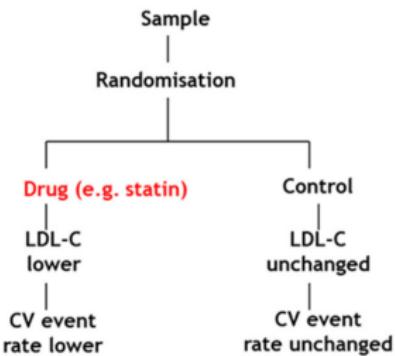
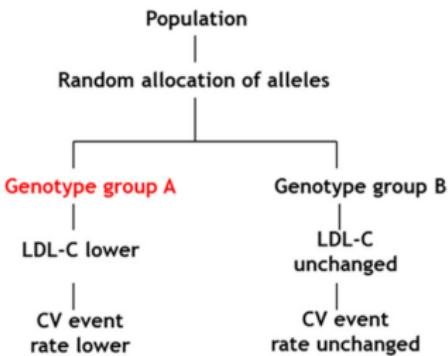
Conventional Trial**Mendelian randomisation**

Figure 3 Comparison of a conventional trial with a Mendelian randomisation study. This illustrates the analogy between a conventional randomised controlled trial and a Mendelian randomisation study. CV, cardiovascular.

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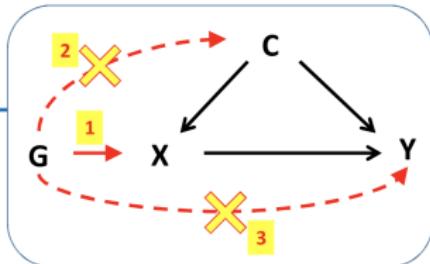
MR의 기본 가정

◆ 전제 1: G → X

- G는 X와 관련성을 가진다: association (eg. GWAS)
- G는 X를 대변할 수 있어야 한다: X의 변이에 대해 유의한 설명력을 가질 것 (F-statistic >10)

$$F\text{-statistic} = \frac{N-K-1}{K} \frac{R^2}{1-R^2}$$

- ✓ N=샘플수, K=SNP 개수, R² regression 식의 값
- ✓ N 클수록, R² 클수록 → larger F



◆ 전제 2: G ↗ X

- G는 G의 기능에 의해 직접 영향을 주는 X 이외의 다른 요인들 (potential C)에 대해 randomization 효과가 있기 때문에, 대부분의 C와 상관관계를 가지지 않는다 (ANOVA로 test).

◆ 전제 3: G는 X를 통해서만 Y와 관련됨

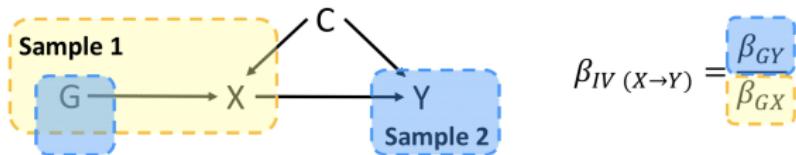
- Pleiotropy (하나의 유전형이 하나 이상의 다른 phenotype에 직접 혹은 간접적으로 영향을 미침)

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Two-Sample MR

- Two-Sample MR

- G-X, G-Y의 자료를 모두 가진 자료가 없을 때도 사용
- 개인단위 자료 없이, GWAS summary data만으로 분석 가능
- 대규모 컨소시엄 자료 활용 가능

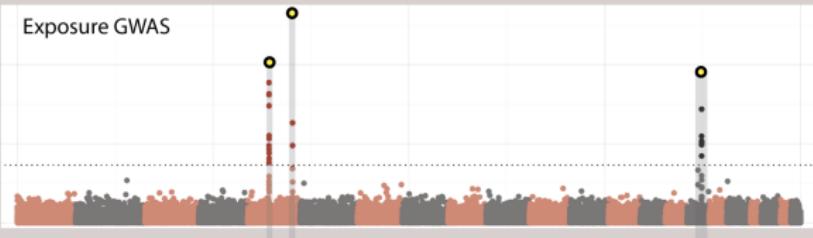
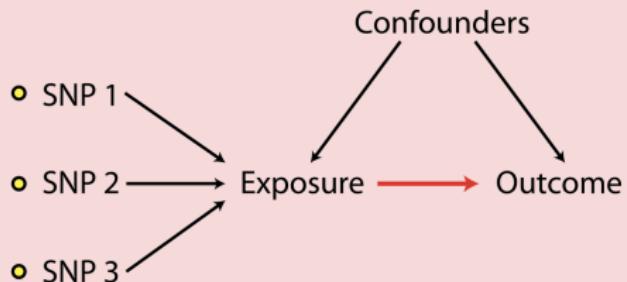


- **MR base:** MR 분석에 필요한 자료와 분석플랫폼을 제공

- Web application 또는 R package ("TwoSampleMR")로 분석 가능

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Objective: Infer the causal effect of the exposure on the outcome

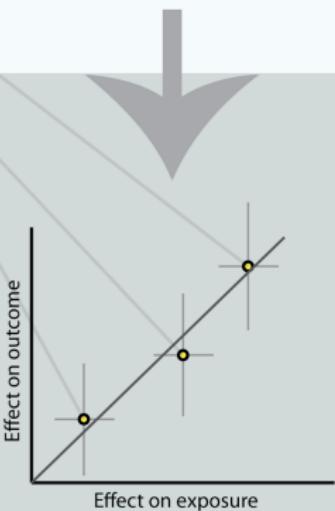


Description

Define instruments: Obtain SNPs that are GWAS significant for the exposure. Ensure that they are independent.

Instruments can be defined from a variety of different sources.

SNP	Exposure GWAS				Outcome GWAS			
	Effect	Effect allele	Other allele	Effect allele frequency	Effect	Effect allele	Other allele	Effect allele frequency
rs123456	0.132	A	G	0.28	0.022	A	G	0.26
rs234567	-0.485	G	T	0.41	-0.056	G	T	0.39
rs345678	0.203	G	C	0.11	0.046	G	C	0.12

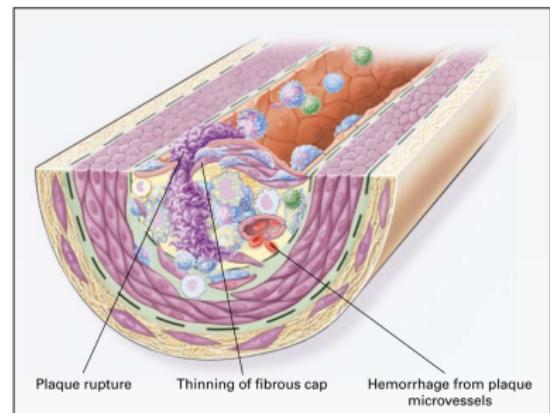


Perform analysis: Using the harmonized data, perform Mendelian randomization analyses and related sensitivity analyses.

The slope of the regression line corresponds to the causal effect of the exposure on the outcome

Atherosclerosis: Leading cause of ischemic stroke

- Artery wall thickens as a result of invasion and accumulation of white blood cells with cholesterol fatty substances, calcium and fibrin.
- Intima of medium and large sized systemic arteries are involved.



Ross R. N Engl J Med 1999

Good predictor or Cause of Atherosclerosis ?

- ① LDL-cholesterol
- ② HDL-cholesterol
- ③ Triglyceride
- ④ Non-HDL-cholesterol

lipoprotein = lipid + apolipoprotein

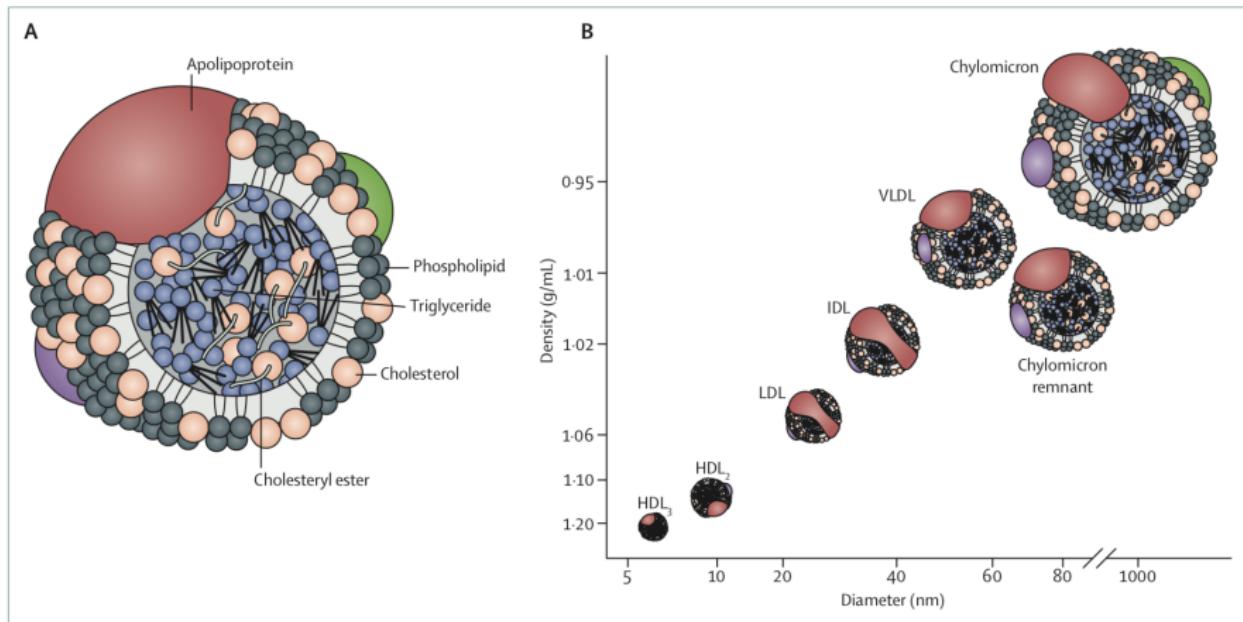


Figure 1: The structural components of lipoproteins (A) and their relation to diameter and density (B)

Adopted from Genest J, Libby P. Lipoprotein Disorders and Cardiovascular Disease. Braunwald's Heart Disease: a textbook of cardiovascular medicine, ninth edition. Elsevier 2012, pp: 975-95. IDL=intermediate-density lipoprotein.

Non-HDL-C captures cholesterol in all atherogenic (apoB-containing) lipoproteins

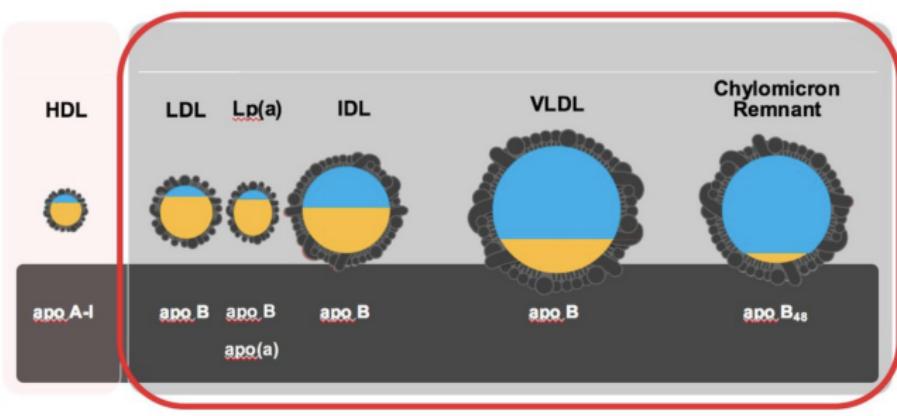


TABLE 400-1 Major Lipoprotein Classes

LIPOPROTEIN	DENSITY, g/mL ^a	SIZE, nm ^b	ELECTROPHORETIC MOBILITY ^c	APOLIPOPROTEINS		OTHER CONSTITUENTS
				MAJOR	OTHER	
Chylomicrons	0.930	75–1200	Origin	ApoB-48	A-I, A-V, C-I, C-II, C-III, E	Retinyl esters
Chylomicron remnants	0.930–1.006	30–80	Slow pre-β	ApoB-48	A-I, A-V, C-I, C-II, C-III, E	Retinyl esters
VLDL	0.930–1.006	30–80	Pre-β	ApoB-100	A-I, A-II, A-V, C-I, C-II, C-III, E	Vitamin E
IDL	1.006–1.019	25–35	Slow pre-β	ApoB-100	C-I, C-II, C-III, E	Vitamin E
LDL	1.019–1.063	18–25	β	ApoB-100		Vitamin E
HDL	1.063–1.210	5–12	α	ApoA-I	A-II, A-IV, A-V, C-III, E	LCAT, CETP, paroxonase
Lp(a)	1.050–1.120	25	Pre-β	ApoB-100	Apo(a)	Oxidized phospholipids

^aThe density of the particle is determined by ultracentrifugation. ^bThe size of the particle is measured using gel electrophoresis. ^cThe electrophoretic mobility of the particle on agarose gel electrophoresis reflects the size and surface charge of the particle, with β being the position of LDL and α being the position of HDL.

Note: All of the lipoprotein classes contain phospholipids, esterified and unesterified cholesterol, and triglycerides to varying degrees.

Abbreviations: CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; Lp(a), lipoprotein A; VLDL, very-low-density lipoprotein.

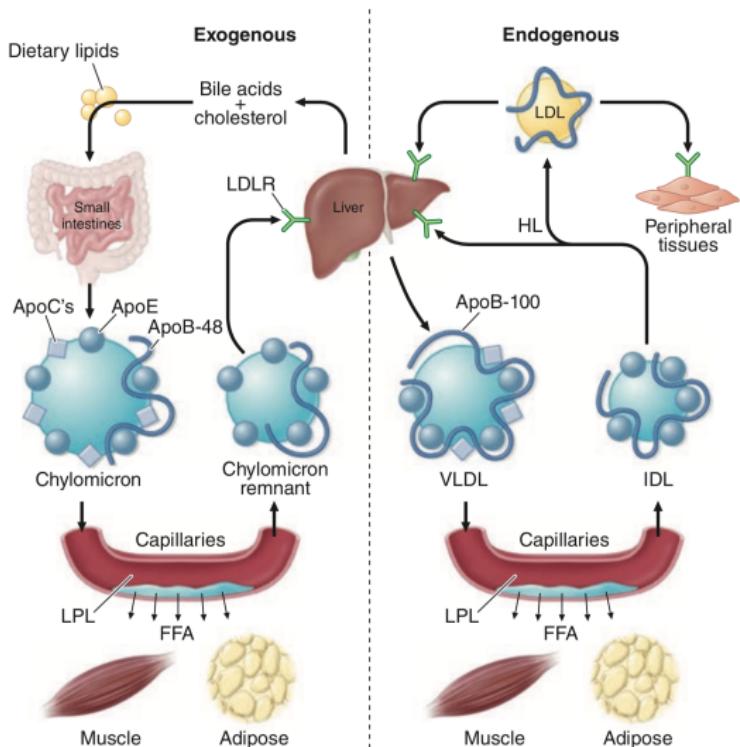
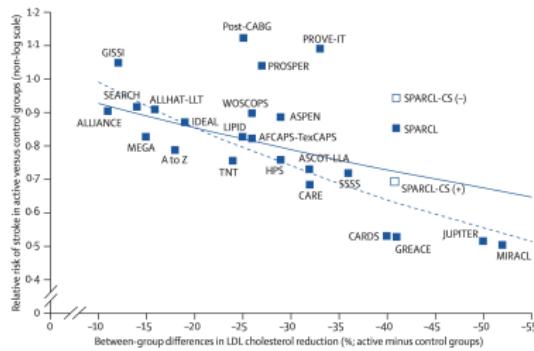


FIGURE 400-2 The exogenous and endogenous lipoprotein metabolic pathways. The exogenous pathway transports dietary lipids to the periphery and the liver. The endogenous pathway transports hepatic lipids to the periphery. FFA, free fatty acid; HL, hepatic lipase; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; VLDL, very-low-density lipoprotein.

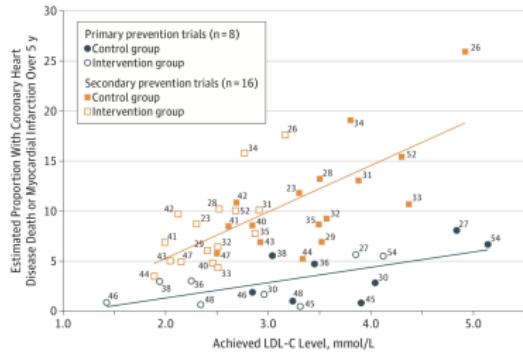
Dose-response relationship in Stroke/IHD and LDL-C



Estimates of relative risk reduction

- 10% LDL reduction: relative risk reduction 7.5% (2.3-12.5) overall
relative risk reduction 13.5% (7.7-18.8) for primary prevention of stroke
- 1 mmol/l (39 mg/dl) LDL reduction: relative risk reduction 21.1% (6.3-33.5) overall
relative risk reduction 35.9% (21.7-47.6) for primary prevention of stroke

Figure 4. Association Between Achieved Low-Density Lipoprotein Cholesterol (LDL-C) and Major Coronary Event Rates From 24 Trials of Established Interventions That Lower LDL-C Predominantly Through Upregulation of LDL Receptor Expression



Amarenco et al. Lancet Neurol 2009;8:453-463; Silverman MG et al. JAMA 2016

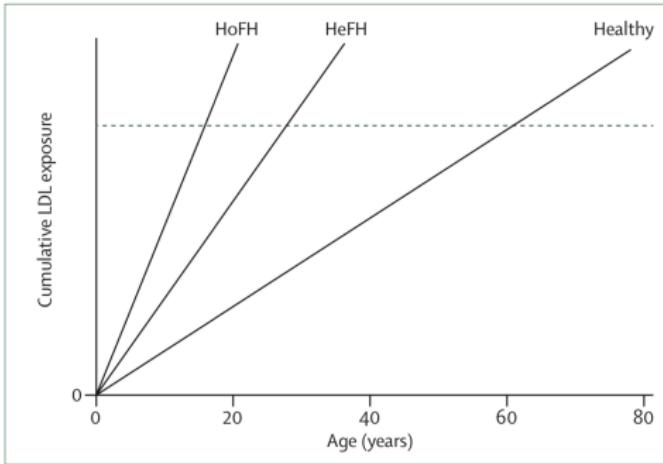
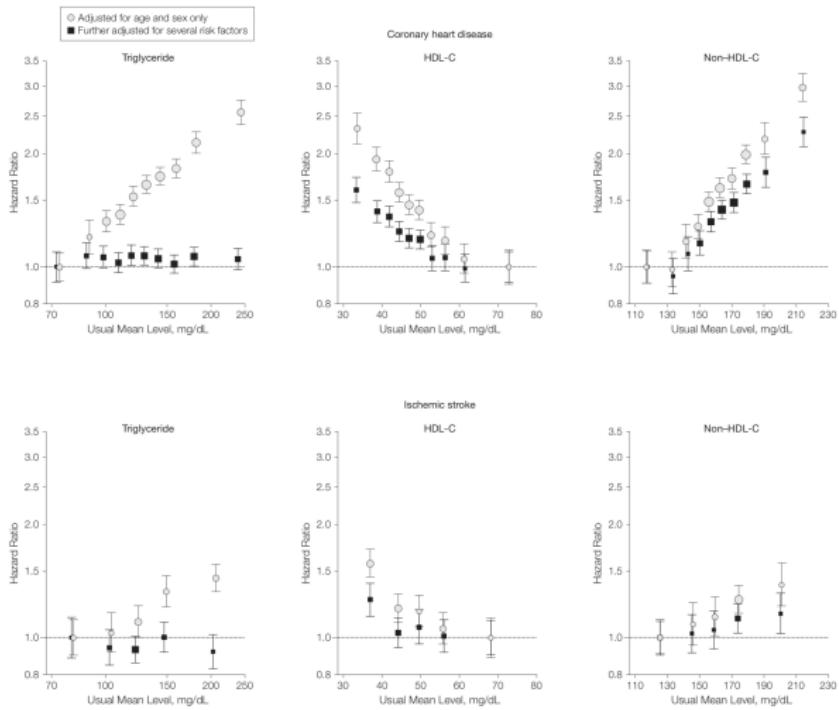


Figure 2: Approximate age of onset of atherosclerotic symptoms for those with homozygous familial hypercholesterolaemia or heterozygous familial hypercholesterolaemia, and those without inherited defects of the LDL-receptor

Adopted from Horton and colleagues.⁶ HoFH=homozygous familial hypercholesterolaemia. HeFH=heterozygous familial hypercholesterolaemia.

Figure 1. Hazard Ratios for Coronary Heart Disease or Ischemic Stroke Across Quantiles of Usual Triglyceride, HDL-C, and Non-HDL-C Levels

Analyses for coronary heart disease were based on 302 430 participants (involving 12 785 cases) from 68 studies. Analyses for ischemic stroke were based on 173 312 participants (involving 2534 cases) from 32 studies. Regression analyses were stratified, where appropriate, by sex and trial group. Values with further adjustments

The statin era

Without background treatment with statins, fibrates were seen to reduce non-fatal myocardial infarction, and niacin to reduce both non-fatal myocardial infarction and stroke. However, in the modern era when treatment with statins is standard, this effect has not been apparent (fig 4). Attempts at risk reduction through these treatments to increase high density lipoprotein levels on top of statin treatment have been unsuccessful so far.

OPEN

Role of Blood Lipids in the Development of Ischemic Stroke and its Subtypes

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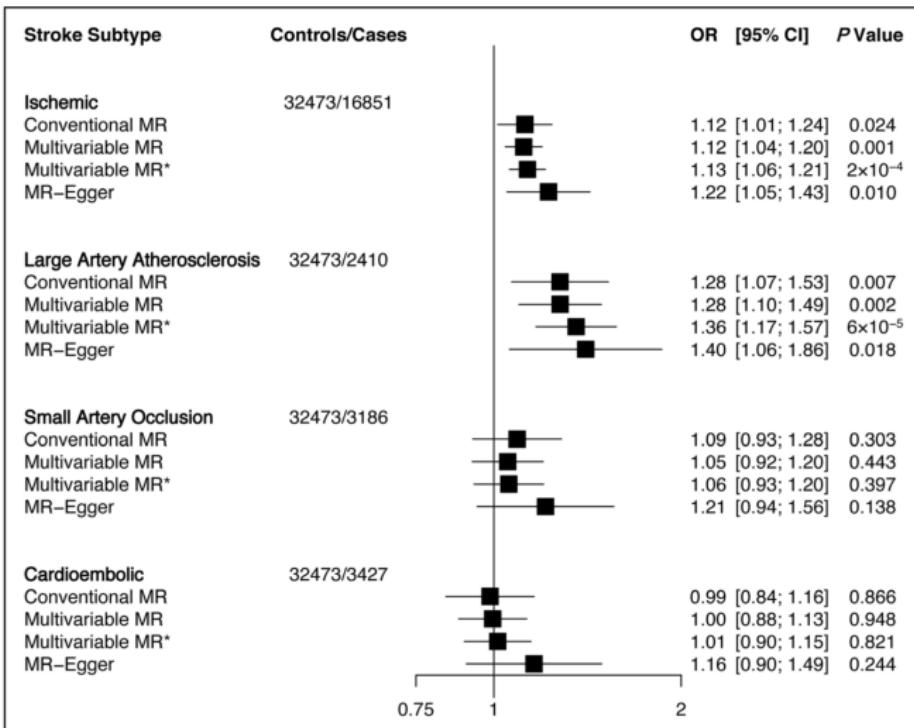


Figure 1. Association of low-density lipoprotein cholesterol with ischemic stroke and subtypes using different Mendelian randomization

Hindry G et al Stroke 2018

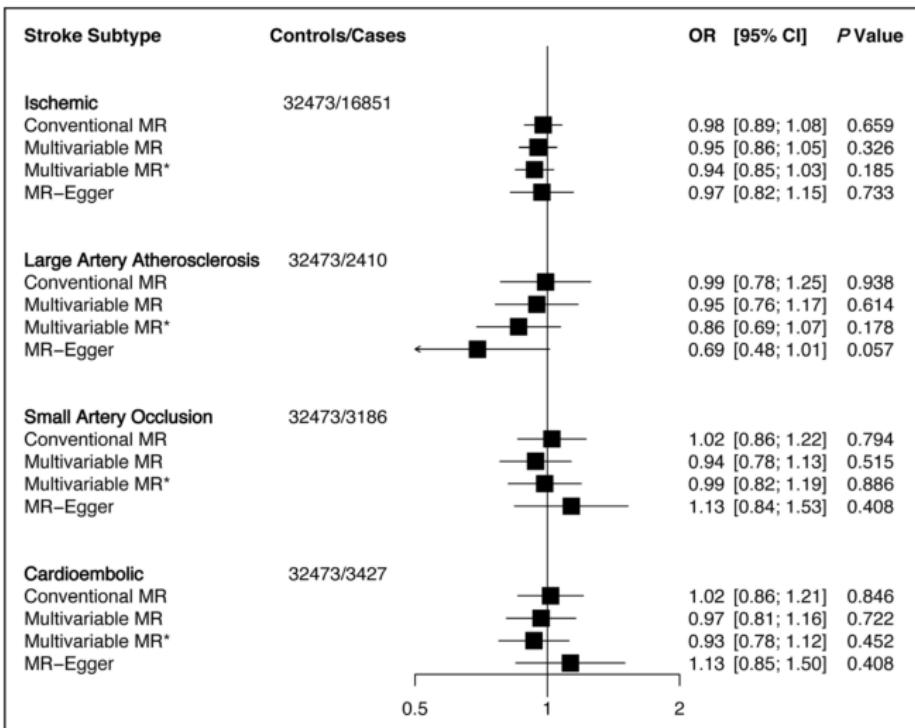


Figure 3. Association of triglycerides with ischemic stroke and subtypes using different Mendelian randomization (MR) analyses. Odds

Hindry G et al Stroke 2018

Published Ahead of Print on February 20, 2019 as 10.1212/WNL.0000000000007091

ARTICLE

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Relative effects of LDL-C on ischemic stroke and coronary disease

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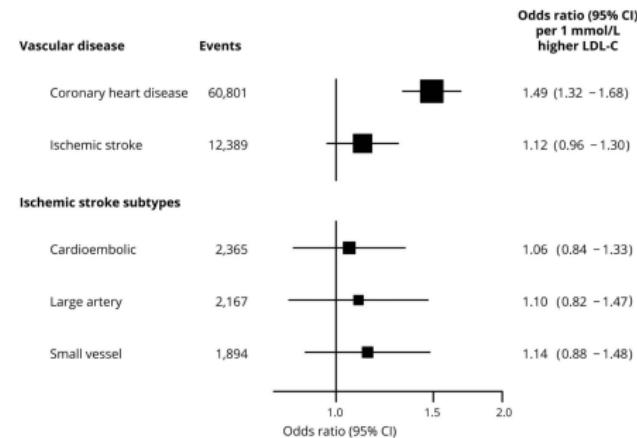
Elsa Valdes-Marquez, PhD, Sarah Parish, DPhil, Robert Clarke, FRCP, Traianu Stari, PhD, and Bradford B. Worrall, MD, METASTROKE Consortium of the ISGC and Jemma C. Hopewell, PhD

Neurology® 2019;92:e1-e12. doi:10.1212/WNL.0000000000007091

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Figure 2 Effects of genetically determined low-density lipoprotein cholesterol (LDL-C) on vascular disease and ischemic stroke subtypes



JAMA | Original Investigation

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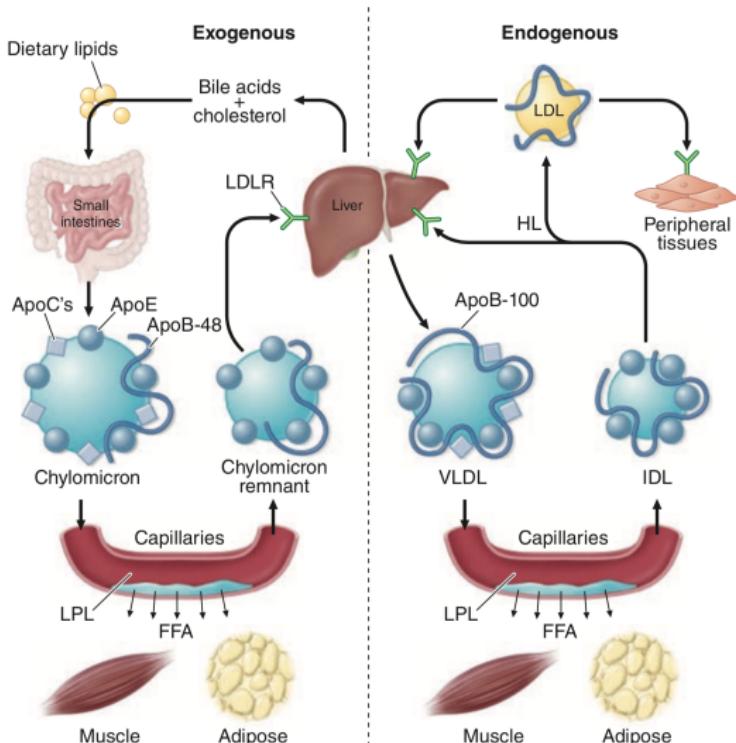
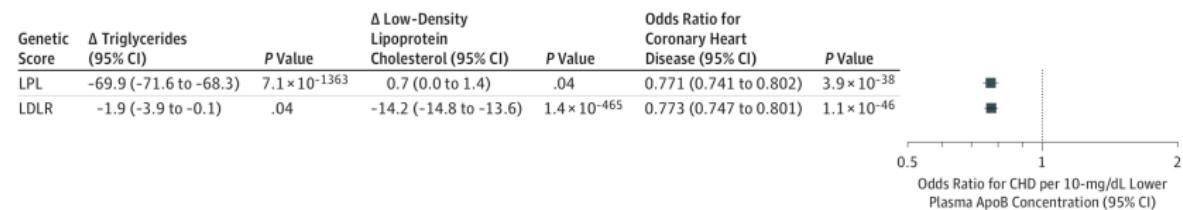


FIGURE 400-2 The exogenous and endogenous lipoprotein metabolic pathways. The exogenous pathway transports dietary lipids to the periphery and the liver. The endogenous pathway transports hepatic lipids to the periphery. FFA, free fatty acid; HL, hepatic lipase; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; VLDL, very-low-density lipoprotein.

Methods

- Calculation of genetic score of LPL and LDL-R
- Genetic score - TG/LDL-C/ApoB and CHD (Linear and logistic regression)
- Associations of each score with risk of CHD was scaled for a common 10-mg/dL lower level of ApoB-containing lipoproteins
- The point estimates derived from the individual participant data and the summary data were then combined across studies in a fixed-effect inverse variance-weighted meta-analysis

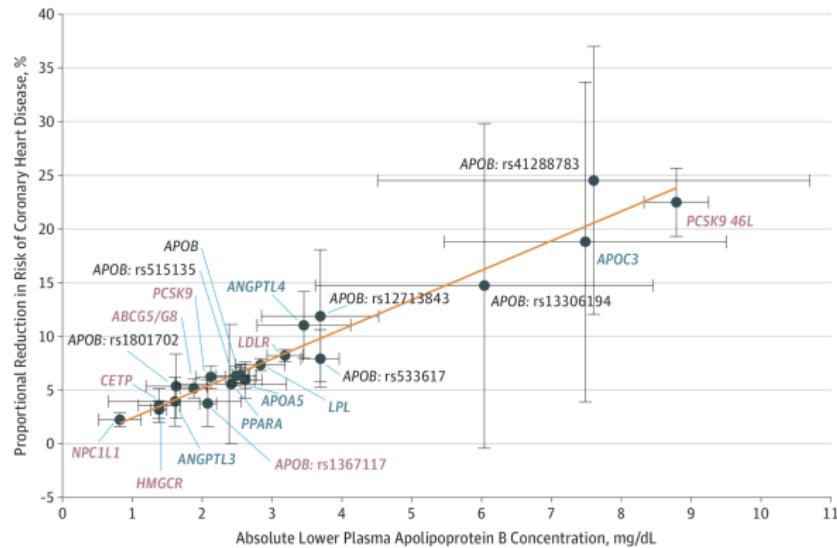
Figure 1. Associations Between the Lipoprotein Lipase (*LPL*) and LDL Receptor Gene (*LDLR*) Genetic Scores With Triglycerides, Low-Density Lipoprotein Cholesterol (LDL-C), and Risk of Coronary Heart Disease (CHD) per 10-mg/dL Lower Concentration of Apolipoprotein B (ApoB)-Containing Lipoproteins



Triglycerides are carried in plasma by ApoB-containing triglyceride-rich lipoproteins while cholesterol is carried predominantly by ApoB-containing low-density lipoproteins. Changes in plasma triglycerides and LDL-C concentration are thus markers of the corresponding changes in the concentration of the ApoB-containing lipoproteins that transport these lipids. Variants in the *LPL* gene that increase *LPL* activity are associated with lower triglycerides and a corresponding lower ApoB concentration, while variants in the *LDLR* gene that increase activity of the LDL receptor are associated with lower LDL-C and a corresponding lower ApoB. The figure shows that for each 10-mg/dL lower plasma ApoB concentration associated with variants in the *LPL* score, there is a corresponding 69.9-mg/dL lower triglyceride level, no change in LDL-C, and a lower risk of CHD (odds ratio, 0.771 [95% CI, 0.741-0.802]). By contrast,

for the same 10-mg/dL lower plasma ApoB concentration associated with variants in the *LDLR* score, there is a corresponding 14.1-mg/dL lower LDL-C level, no change in triglycerides, and a similar lower risk of CHD (odds ratio, 0.773 [95% CI, 0.747-0.801]). Therefore, despite being associated with changes in different lipids, the *LPL* and *LDLR* scores were associated with similar lower risk of CHD for the same lower plasma ApoB concentration. The data presented are for the associations of the *LPL* and *LDLR* genetic scores with risk of CHD per 10-mg/dL decrease in ApoB-containing lipoproteins in all 654 783 participants included in the study. The associations of either score with changes in triglycerides and LDL-C per 10-mg/dL lower level of ApoB-containing lipoproteins are from up to 305 699 participants enrolled in the Global Lipid Genetics Consortium. Boxes represent effect size estimates and lines represent 95% CIs.

Figure 3. Log-Linear Association Between Absolute Differences in Apolipoprotein B (ApoB) and Lower Risk of Coronary Heart Disease (CHD)



The associations of each genetic variant with ApoB concentration is plotted against its unadjusted association with CHD, expressed as a proportional lower risk (calculated as $[1-OR_{CHD}] \times 100$). Variants in the genes that encode the targets of therapies that lower triglycerides through the LPL pathway are marked with blue labels, and variants in the genes that encode the targets of therapies that lower LDL-C through upregulation of the LDL receptor are marked by red labels. Circles represent the associated absolute change in ApoB

and corresponding proportional lower risk of CHD for each variant. The horizontal lines through each circle represent ± 1 standard errors for the associated absolute change in ApoB for each variant; and the vertical line through each circle represents ± 1 standard errors for the associated proportional lower risk of CHD. Associations with CHD were measured in all 654 783 participants included in the study; associations with ApoB were measured in a meta-analysis of 14 studies including up to 84 324 participants.

Table 3. Multivariable Mendelian Randomization Analysis of the Association Between Plasma Triglycerides, LDL-C, and ApoB With the Risk of CHD^a

Analysis	Variables	Odds Ratio for CHD (95% CI)	P Value
Association of 10-mg/dL lower ApoB with risk of CHD	ApoB	0.770 (0.760-0.781)	1.42E-170
Association of 10-mg/dL lower LDL-C with risk of CHD	LDL-C	0.846 (0.833-0.858)	8.16E-77
Association of 50-mg/dL lower triglycerides with risk of CHD	Triglycerides	0.815 (0.785-0.846)	1.37E-18
Association of 10-mg/dL lower LDL-C and 50-mg/dL lower triglycerides with risk of CHD included in same model	LDL-C	0.862 (0.849-0.875)	6.92E-65
	Triglycerides	0.876 (0.850-0.902)	1.36E-14
Association of 10-mg/dL lower LDL-C, 50-mg/dL lower triglycerides, and 10-mg/dL lower ApoB with risk of CHD included in same model	ApoB	0.761 (0.723-0.798)	7.51E-20
	LDL-C	1.010 (0.967-1.055)	.19
	Triglycerides	1.014 (0.965-1.065)	.19

Abbreviations: ApoB, apolipoprotein B; CHD, coronary heart disease; LDL-C, low-density lipoprotein cholesterol.

^a Data presented are derived from a multivariable meta-regression analysis of 186 genetic variants, including the 5 variants included in the *LPL* score, 3 variants included in the *LDLR* score, and 178 variants associated with either triglycerides, LDL-C, or both at genome-wide significance as reported by the Global Lipids Genetics Consortium. Effect sizes for the associated risk of CHD are reported per 10-mg/dL lower ApoB concentration, per 10-mg/dL lower LDL-C level, or per 50-mg/dL lower triglyceride level (because dividing triglyceride concentration by 5 estimates the cholesterol content carried by triglyceride-rich ApoB-containing lipoproteins as estimated by the Friedewald formula). In these analyses, the dependent variable was the effect estimate for risk of CHD in all 654 783 participants included in the study for each variant and the independent variables were the effect estimates for the associated changes in plasma triglycerides, LDL-C, and ApoB, measured in up to 305 699 participants in Global Lipids Genetics Consortium for each variant. The analysis

was weighted by the inverse squared standard error of the associated risk of CHD for each variant and forced to pass through the origin. For example, in multivariable mendelian randomization analyses involving these 186 genetic variants, both triglycerides (odds ratio [OR], 0.876 per 50-mg/dL lower triglycerides) and LDL-C (OR, 0.862 per 10-mg/dL lower LDL-C) were independently associated with a lower risk of CHD at genome-wide level of significance. By contrast, when ApoB was included in the multivariable mendelian randomization analyses, the associations with CHD for both triglycerides (OR, 1.014 per 50-mg/dL lower triglycerides) and LDL-C (OR, 1.010 per 10-mg/dL lower LDL-C) became null, but the association per 10-mg/dL lower ApoB remained unchanged (OR, 0.761 per 10-mg/dL lower ApoB). The unadjusted associations with triglycerides, LDL-C, ApoB, and CHD for each variant included in the analysis are provided in eTable 7 in the *Supplement*. Additional multivariable meta-regression analyses for various combinations of these variants is provided in eTable 8 in the *Supplement*.

Key Points

Question What is the clinical benefit of lowering plasma triglyceride levels compared with lowering low-density lipoprotein cholesterol levels?

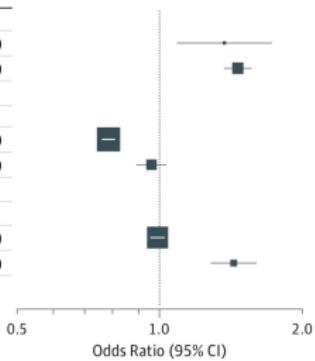
Findings In mendelian randomization analyses involving 654 783 participants, triglyceride-lowering variants in the lipoprotein lipase gene and low-density lipoprotein cholesterol (LDL-C)-lowering variants in the LDL receptor gene were associated with similar lower risk of coronary heart disease per 10-mg/dL lower level of apolipoprotein B (ApoB)-containing lipoproteins (odds ratios of 0.771 and 0.773, respectively).

Meaning The clinical benefit of lower triglyceride levels was similar to the clinical benefit of lower LDL-C levels per unit difference in ApoB and may be related to the absolute reduction in ApoB-containing lipoprotein particles.

Summary

Figure. Comparison of Observational Estimates and Mendelian Randomization Estimates of the Association of Low-Density Lipoprotein (LDL) Cholesterol, High-Density Lipoprotein (HDL) Cholesterol, and Triglycerides With Coronary Heart Disease

Analysis	Source	Odds Ratio (95% CI)
LDL cholesterol		
Observational	ERFC ²	1.37 (1.09-1.73)
Mendelian randomization	Do et al ³	1.46 (1.37-1.56)
Test for heterogeneity: $P = .60$		
HDL cholesterol		
Observational	ERFC ²	0.78 (0.76-0.81)
Mendelian randomization	Do et al ³	0.96 (0.89-1.03)
Test for heterogeneity: $P < .01$		
Triglycerides		
Observational	ERFC ²	0.99 (0.96-1.03)
Mendelian randomization	Do et al ³	1.43 (1.28-1.60)
Test for heterogeneity: $P < .01$		



Observational estimates are derived from the Emerging Risk Factors Collaboration (ERFC).² Mendelian randomization estimates are derived from Do et al³ based on an analysis of 185 genetic variants that alter plasma lipids and mutually adjusted for other lipid fractions (eg HDL cholesterol and triglycerides for LDL cholesterol). A formal test of heterogeneity (Cochran Q test) shows that the observational and mendelian randomization causal estimates are consistent for LDL cholesterol but not so for HDL cholesterol or triglycerides.