Mendelian Randomisation studies and LDL causality

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Introduction

• Molecular genetics is progressively entering clinical practice and it is affecting patients’ management.

• Most of our current knowledge derives from the application to molecular diagnosis of the findings of the pivotal studies that have unveiled the genes that cause the so-called monogenic diseases.

• Thanks to these studies we have learnt that disorders such as hypertrophic cardiomyopathy, dilated cardiomyopathy or long QT syndrome arise from a large spectrum of genetic defects and that the type of DNA abnormality is not only a molecular curiosity but also bears prognostic and therapeutic implications.

• The next challenge for molecular geneticists is to investigate the role of DNA variants or single nucleotide polymorphisms in determining the predisposition to develop more complex phenotypes such as ischaemic heart disease, hypertension and heart failure.
**Gregor Mendel**
(1822–1884)

Responsible for the Laws governing Inheritance of Traits

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**Gregor Johann Mendel**

- Austrian monk
- Studied the inheritance of traits in *pea plants*
- Developed the laws of inheritance
- Mendel's work was not recognized until the turn of the 20th century
Genetic Terminology

- **Trait** - any characteristic that can be passed from parent to offspring
- **Heredity** - passing of traits from parent to offspring
- **Genetics** - study of heredity

What is Mendelian Randomisation?

- Approach to test for a causal effect from observational data in the presence of certain confounding factors.
- Uses the measured variation of genes of known function, to bound the causal effect of a modifiable exposure (environment) on a phenotype (disease).
- Fundamental idea is that the genotypes are randomly assigned (due to meiosis).
- This allows them to be used as an instrumental variable.
Atherosclerosis and LDL

- Atherosclerotic cardiovascular disease (ASCVD) and its clinical manifestations, such as myocardial infarction (MI) and ischaemic stroke, are the leading cause of morbidity and mortality throughout the world. Multiple exposures have been reported to be associated with an increased risk of cardiovascular events.

- The most extensively studied of these exposures by far is low-density lipoprotein (LDL).

- Despite this extensive body of evidence, however, some still express scepticism of the causal nature of the relationship between DL and the development of ASCVD.

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**INTERHEART Study**

LDL accounted for ~50% of the Population Attributable Risk

- Alcohol Intake
- Smoking
- Diabetes
- Hypertension
- Psychosocial Stress
- Abdominal Obesity
- Vegetable and Fruit consumption
- Exercise

High Global CV Risk

Accelerated Atherosclerosis and CV disease

Slide courtesy of MI Chapman
The Evidence Reviewed

• To avoid selection bias the **totality of evidence** was evaluated from separate meta-analyses of prospective epidemiologic studies, Mendelian randomization and other genetic studies, together with randomized clinical trials for causality of LDL in ASCVD

• The database included *more than 200 studies involving over 2 million participants with over 20 million person-years of follow-up and more than 150,000 cardiovascular events*

LDL vs. LDL Cholesterol

• LDL is the main apolipoprotein B-containing lipoprotein
• LDL-C is the total amount of cholesterol contained in LDL particles, and is usually calculated
• Under most conditions, LDL-C concentration and LDL particle number are highly correlated
• **LDL particles comprise ~ 90% of circulating apoB-containing lipoproteins**
Evidence from Inherited Disorders of Lipid Metabolism

**Familial Hypercholesterolaemia (FH)**

- **Clinical diagnosis**
  - LDL cholesterol mmol/L mg/dL
  - 20 770
  - 15 580
  - 13 500
  - 10 390
  - 5 190

- **Homozygous FH**
- **Heterozygous FH**
- **Common or polygenic hypercholesterolemia**

The most frequently mutated gene in FH is the LDL receptor gene.
LDL-C Burden With or Without FH as a Function of Age

Evidence from Prospective Epidemiologic Studies

Plasma LDL-C concentration is strongly and log-linearly associated with a dose-dependent increase in risk of ASCVD events.

Mendelian Randomization
A naturally randomized trial which largely avoids confounding by other factors.
Continuous, dose-dependent and log-linear causal association between the magnitude of the absolute change in LDL-C level and the lifetime risk of CHD.

European Heart Journal. doi:10.1093/eurheartj/ehx144
Mendelian Randomization Studies

• Meta-analyses of Mendelian randomization studies involving >300,000 participants and 80,000 CHD cases provide compelling evidence that LDL is causally associated with the risk of ASCVD.

• The causal effect of LDL on ASCVD is largely independent of the mechanism by which LDL is ‘lowered’.

What is Mendelian Randomisation?: An example

• Katan MB (1986): Apolipoprotein E isoforms, serum cholesterol, and cancer.
• Do low serum cholesterol levels increase cancer risk?
• But maybe both cancer risk and cholesterol levels are affected by diet (confounders)
• Or latent tumours cause the lower cholesterol level (reverse causation)
• But patients with Abetalipoproteinemia (inability to absorb cholesterol) - did not appear predisposed to cancer
• Led Katan to idea of finding a large group genetically predisposed to lower cholesterol levels
• This is Mendelian Randomisation.
• Note this does not require that the genetic variants are direct determinants of health. But, uses the association to improve inferences of the effects of modifiable environmental risks on health.
What is Mendelian Randomisation?: An example

• Apolipoprotein E (ApoE) gene was known to affect serum cholesterol, with the ApoE2 variant being associated with lower levels.

• Many individuals carry ApoE2 variant and so have lower cholesterol levels from birth.

• Since genes are randomly assigned during meiosis (due to recombination), ApoE2 carriers should not be different from ApoE carriers in any other way (diet, etc.), so there is no confounding via the genome - note these assumptions.

• Therefore if low serum cholesterol is really causal for cancer, the cancer patients should have more ApoE2 alleles than the controls - if not then the levels would be similar in both groups.

How could Mendelian randomisation studies answer the questions of causality?

• MR is a technique for judging the causal impact of a risk factor on an outcome from observational data using genetic variants.

• Genetic evidence for a link between hypercholesterolemia and CHD risk has a long history.

• New data resources and new methodological approaches have given fresh insight into this technique (Multivariate MR, MR Egger, Weighted median method)
Lessons from MR studies in dyslipidemia

- LDL-c abnormalities are direct causes of CAD
- Benefits of low LDL-c increase with time (The effect estimate from MR is 3.5 times greater than that of statin trials)
- Early detection and may be treatment is better
- There is a causal relationship between high Lp (a) and CAD
- The causal effect of high Trigs and low HDL is less clear
Evidence from Randomized Controlled Trials

Reducing plasma LDL-C levels with a statin leads to dose-dependent reduction in the risk of major ASCVD events that is proportional to the absolute magnitude of the reduction in achieved LDL-C.

European Heart Journal. doi:10.1093/eurheartj/ehx144
Evidence from Randomized Controlled Trials
These trials are with pharmacological agents that involve the LDL receptor
Site of Action of LDL-lowering Therapies

Evidence from IVUS Studies
Progression of coronary atherosclerotic plaque volume can be arrested at achieved LDL-C levels of ~1.8 mmol/L (70 mg/dL)
Expected proportional risk reduction based on pre-treatment low-density lipoprotein cholesterol (LDL-C), absolute magnitude of LDL-C reduction, and total duration of therapy

<table>
<thead>
<tr>
<th>Baseline LDL-C (mmol/L)</th>
<th>Absolute reduction LDL-C (mmol/L)</th>
<th>Duration of treatment exposure [expected proportional risk reduction (%)]: 5 years</th>
<th>10 years</th>
<th>20 years</th>
<th>30 years</th>
<th>40 years</th>
<th>Guideline recommended treatment</th>
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Expected short-term absolute risk reduction and number needed to treat based on baseline absolute risk of cardiovascular disease and pre-treatment low-density lipoprotein cholesterol (LDL-C) with 5 years of treatment to lower LDL-C

<table>
<thead>
<tr>
<th>10-year absolute risk of CVD (%)</th>
<th>Baseline LDL-C mmol/L (mg/dL)</th>
<th>LDL-C after 50% reduction mmol/L (mg/dL)</th>
<th>Proportional risk reduction (%)</th>
<th>10-year Absolute risk (%) after 50% LDL-C reduction</th>
<th>ARR (%)</th>
<th>NNT</th>
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### Summary of the Causality Evidence

#### Criteria for Causality: LDL and ASCVD

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Evidence Grade*</th>
<th>Summary of Evidence for LDL</th>
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| Plausibility       | 1               | • LDL and other apo B-containing lipoproteins (VLDL, IDL and Lp(a)) are directly implicated in the *initiation and progression of ASCVD*  
• Experimentally induced elevations in plasma LDL and other apoB-containing lipoproteins *lead to atherosclerosis* in all mammalian species studied. |
| Strength           | 1               | • Monogenic and polygenic-mediated *lifelong elevations in LDL* lead to markedly higher lifetime risk                                                                                                                      |
| Biological gradient| 1               | • Monogenic lipid disorders, prospective cohort studies, Mendelian randomization studies, and randomized intervention trials uniformly demonstrate a *dose-dependent, log-linear association* between the *absolute magnitude of exposure to LDL* and *risk of ASCVD* |
| Temporal sequence  | 1               | • Monogenic lipid disorders and Mendelian randomization studies demonstrate that *exposure to elevated LDL precedes the onset of ASCVD*                                                                          |
| Specificity        | 1               | • Mendelian randomization studies and randomized intervention trials both provide unconfounded randomized evidence that LDL is *associated with ASCVD independent of other risk factors* |
**LDL and ASCVD: Key Findings**

- Cumulative LDL burden determines the initiation and progression of ASCVD.

- There is a dose-dependent, log-linear association between absolute LDL-C level and cardiovascular risk. This association is independent of other cardiovascular risk factors and consistent across the multiple lines of evidence.

- Evidence accrued from >30 randomized trials involving >200,000 individuals and 30,000 cardiovascular events evaluating treatments specifically designed to lower LDL consistently show that reducing LDL-C reduces the risk of cardiovascular events. This benefit is proportional to the absolute reduction in LDL-C.

Low-density lipoprotein (LDL) as a causal factor for atherosclerotic cardiovascular disease: key implications

• Cumulative LDL arterial burden is a central determinant for the initiation and progression of atherosclerotic cardiovascular disease.
• The lower the LDL cholesterol (LDL-C) level attained by agents that primarily target LDL receptors, the greater the clinical benefit accrued.
• Both proportional (relative) risk reduction and absolute risk reduction relate to the magnitude of LDL-C reduction.
• Lowering LDL-C in individuals at high cardiovascular risk earlier rather than later appears advisable, especially in those with familial hypercholesterolaemia.

Implications

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Thank You