Mendelian Randomization analysis to provide support for causal associations on frailty

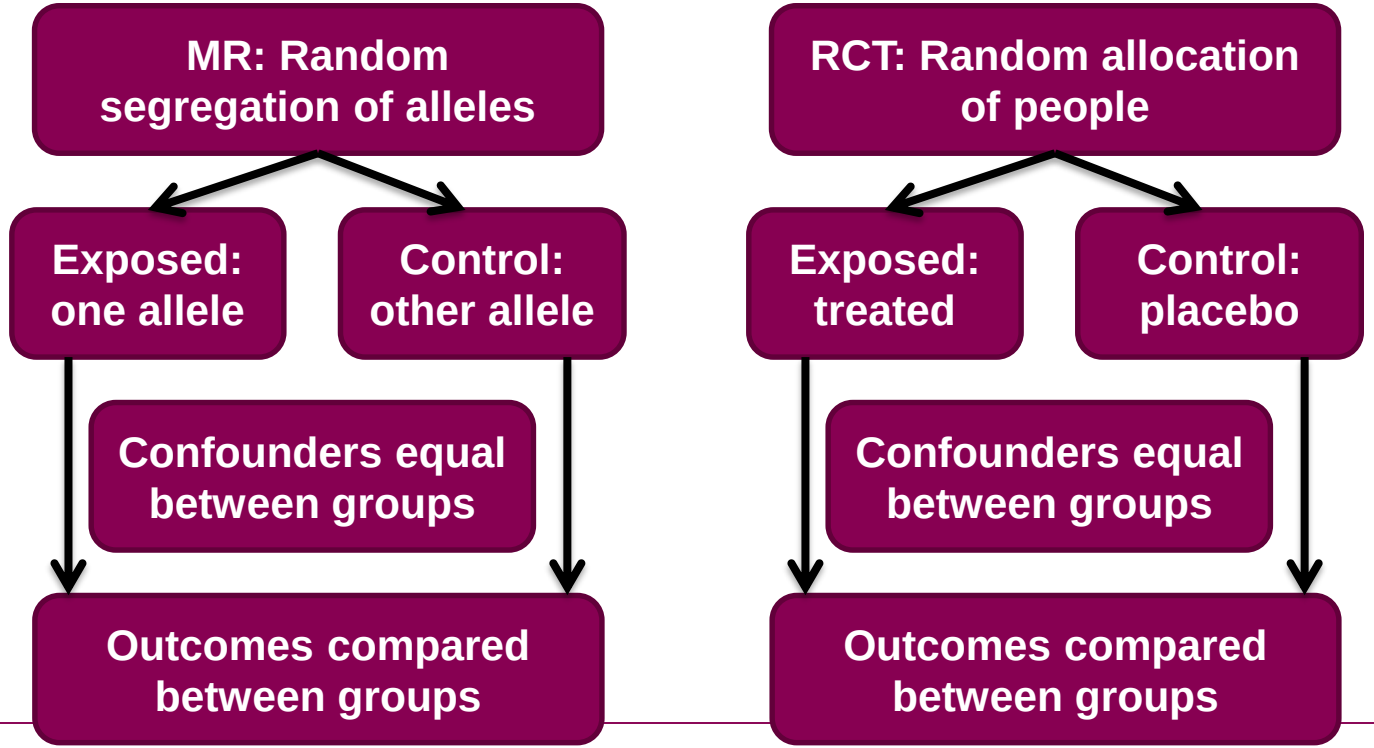
LUND UNIVERSITY POPULATION RESEARCH PLATFORM (LUPOP)
SEMINAR SERIES
March 17, 2022

Sara Hägg
Docent in Molecular Epidemiology
Department of Medical Epidemiology and Biostatistics (MEB)
Karolinska Institutet

Outline

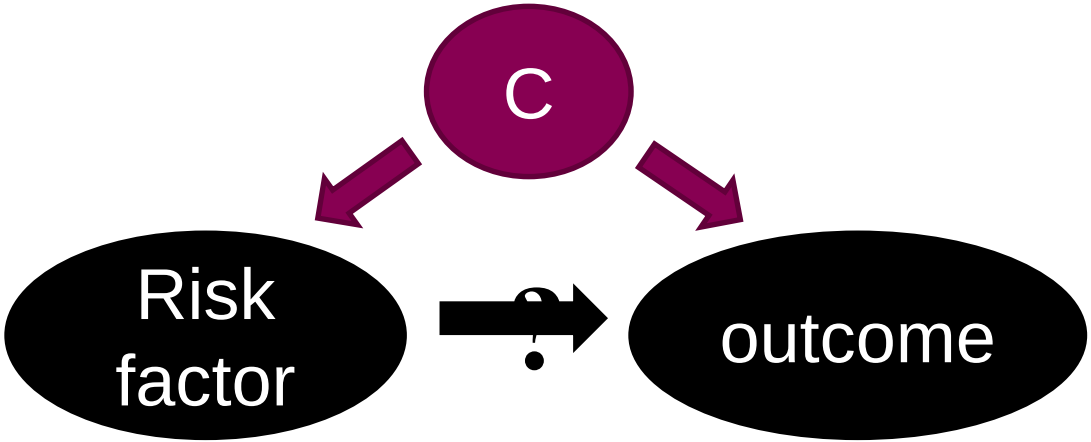
- Introduction to Mendelian Randomization
- Assumptions of Mendelian Randomization
- Introduction to frailty
- Example studies using MR and frailty

Mendelian Randomization & RCT



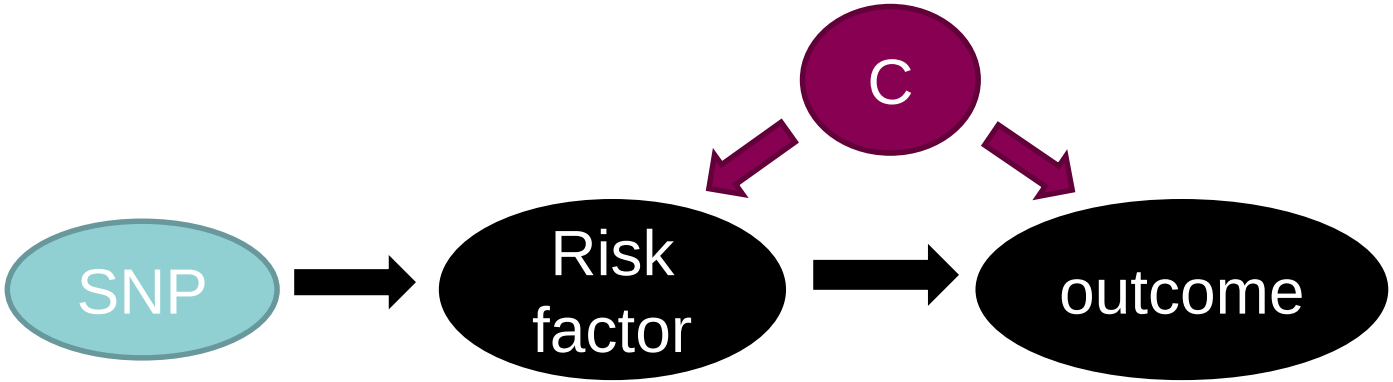
Traditional epidemiology designs

- Observational studies are subject to confounding, selection bias and reverse causation



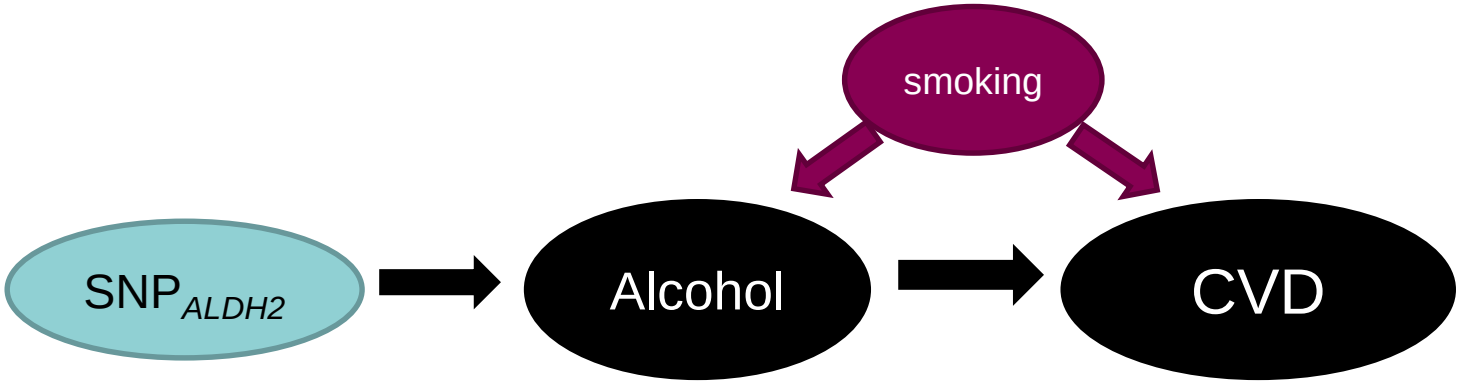
Mendelian randomization design

- Take the advantage of genetic variants as a nonconfounded proxy for the risk factor



Mendelian randomization: example

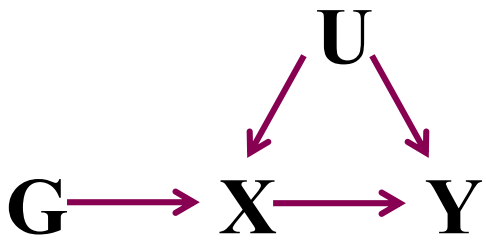
The enzyme aldehyde dehydrogenase is responsible for efficient metabolism of alcohol after it has been oxidized to acetaldehyde. Peak blood acetaldehyde concentrations after drinking alcohol are 18 times higher among people who are homozygous for the null variant allele and five times higher among heterozygous people compared with people with two functioning alleles.



Core assumptions of MR

- An instrumental variable (G) should satisfy the following assumptions:
  1. The IV *G* is robustly associated with the exposure of interest *X*
  2. *G* is independent of confounding factors *U* that confound the association of *X* and the outcome *Y*
  3. *G* is independent of outcome *Y* given *X* and confounding factors *U*

     (no pleiotropy)



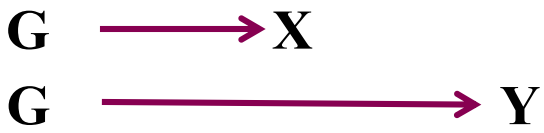
1. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. Statistical Methods in Medical Research 2007

What data can be used?

One-sample

- Exposure and outcome in the same data
- Meta-analyses, e.g., consortium
- Individual level data, e.g., UK Biobank

Two-sample



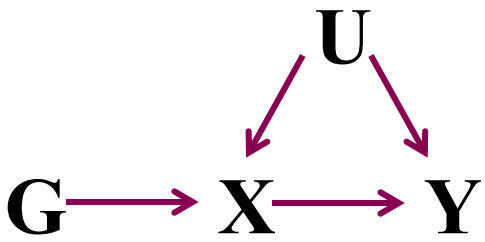
- Exposure and outcome in different data
- Summary level data, e.g., consortium

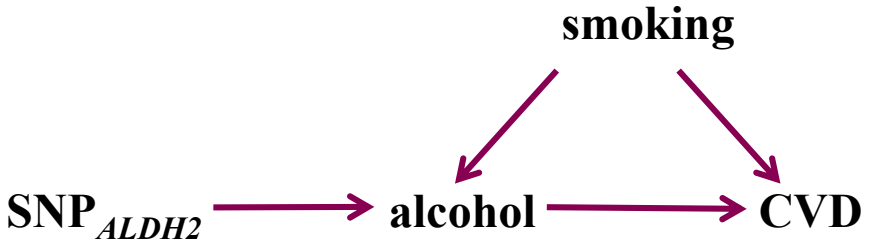
- An instrumental variable (G) should satisfy the following assumptions:
  1. The IV *G* is robustly associated with the exposure of interest *X*

Table 1 | Three key assumptions that must hold for a Mendelian randomisation study to be valid

		Tools to assess plausibility	
Assumption	**Description**	**Single sample**	**Two sample**
Relevance assumption	The genetic variants associate with the risk factor of interest	The partial F statistic and partial r squared, or risk difference	Variants are associated with the risk factor in a large genome-wide study

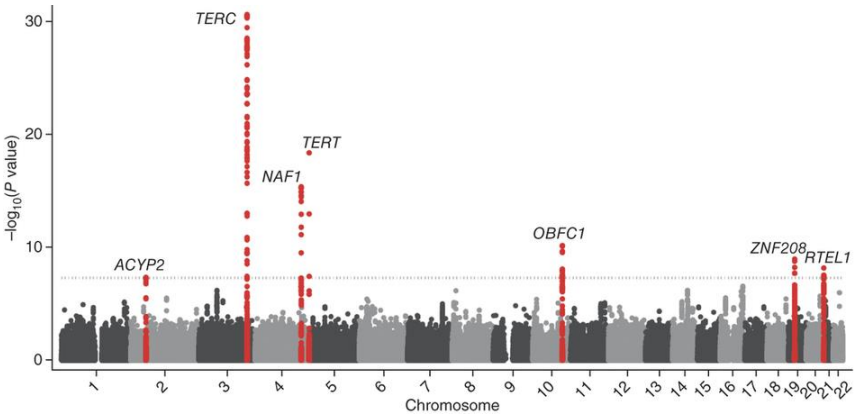
Neil M Davies et al. BMJ 2018;362:bmj.k601





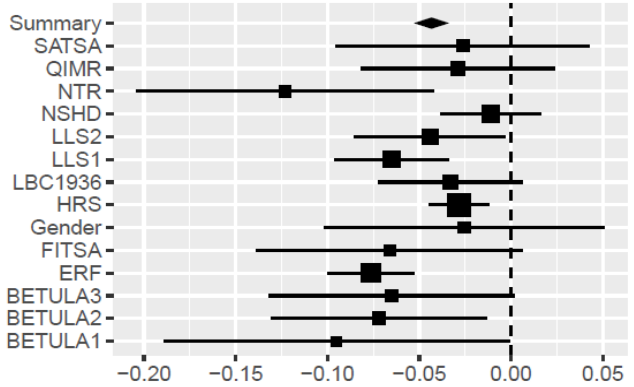
Assumption 1: SNP (G) is robustly associated with exposure (X)

Genetic variants of telomere length



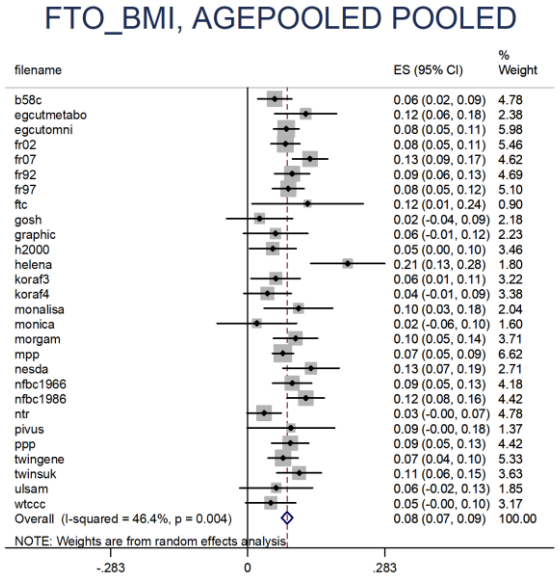
Codd V. *et al*. Nat Genet. 2013, 45.422–427

Genetic risk score (GRS)



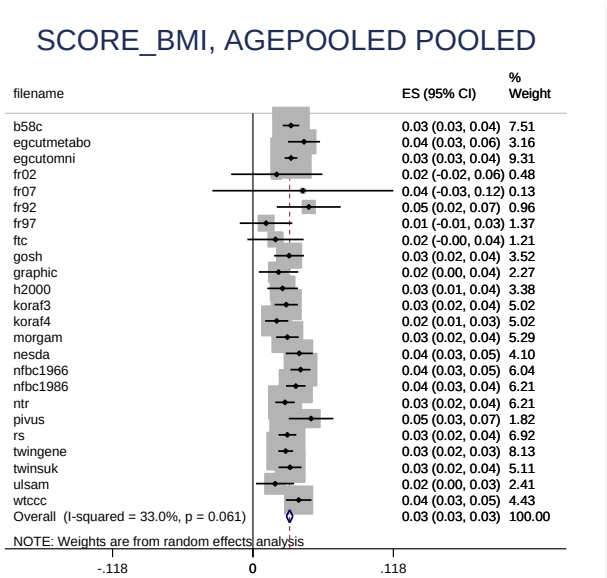
Hägg et al. Transl Psych, 2017

Testing assumption 1: *FTO* vs. SCORE



n=124,527

P-value=9 x 10^{-44}



n=89,995

P-value=7 x 10^{-123}

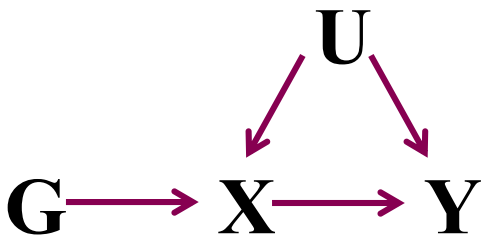
Fall et al, PLoS Med, 2013; Hägg et al. Int J Epidemiol, 2015

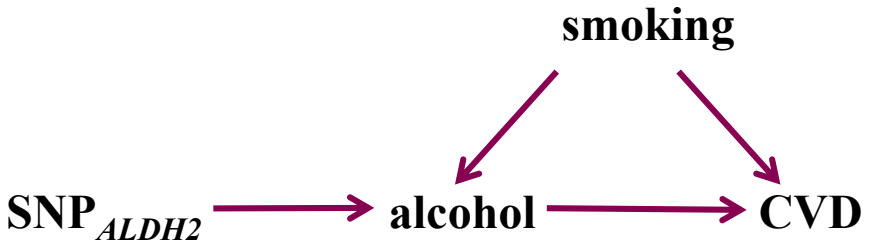
- An instrumental variable (G) should satisfy the following assumptions:
  1. The IV *G* is robustly associated with the exposure of interest *X*
  2. *G* is independent of confounding factors *U* that confound the association of *X* and the outcome *Y*

Table 1 | Three key assumptions that must hold for a Mendelian randomisation study to be valid

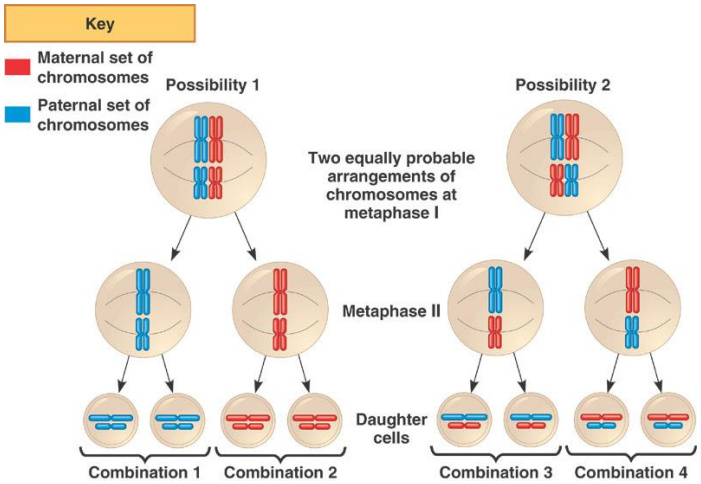
		Tools to assess plausibility	
Assumption	**Description**	**Single sample**	**Two sample**
Relevance assumption	The genetic variants associate with the risk factor of interest	The partial F statistic and partial r squared, or risk difference	Variants are associated with the risk factor in a large genome-wide study
Independence assumption	There are no unmeasured confounders of the associations between genetic variants and outcome	Covariate balance tests and bias component plots. Adjusting for principal components of population stratification	Evidence from large genome-wide association studies on the association of the genetic variants used as instruments with other baseline covariates

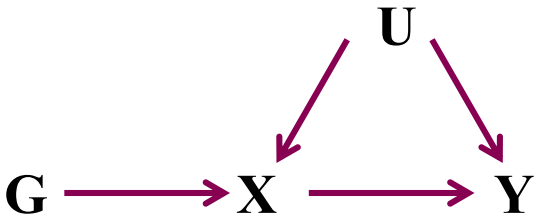
Neil M Davies et al. BMJ 2018;362:bmj.k601





Assumption 2: SNP (G) is independent of confounding factors





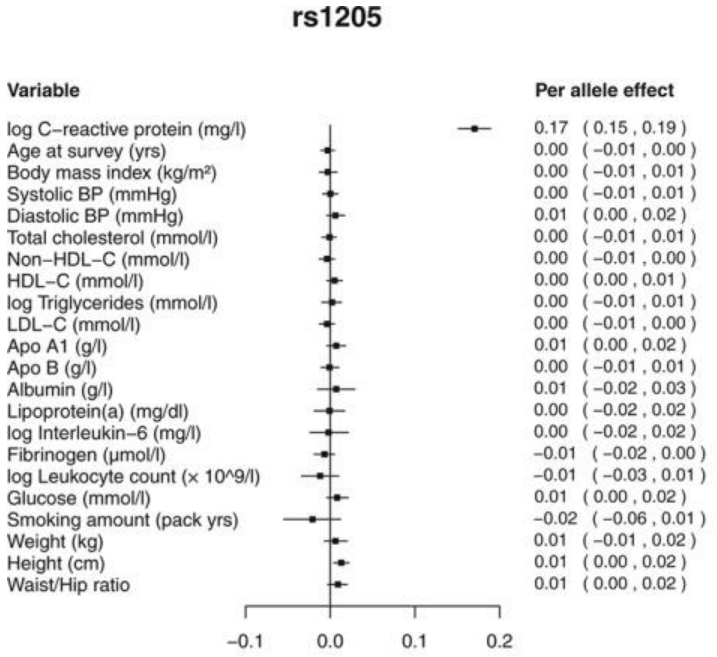
The assignment of the paternal or maternal allele to a gamete is random

This implies that U cannot modify G

Testing assumption 2

Relation between ALDH2 genotype and various characteristics[8]

	Homozygous for null variant	Heterozygous for null variant	Homozygous for functioning variant
Mean alcohol consumption (ml/day)	5.3	15.1	29.2
Mean age (years)	61.3	61.5	60.6
% smokers	48.5	47.9	47.7
Mean HDL cholesterol concentration (mmol/l)	1.24	1.35	1.4
% with hypertension	40.6	37.7	46.9



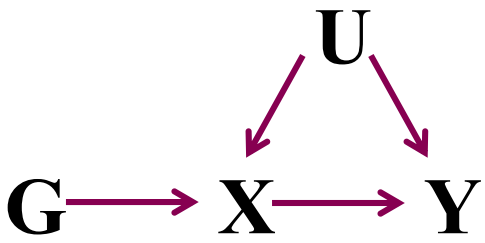
Burgess et al. Epidemiology, 2017

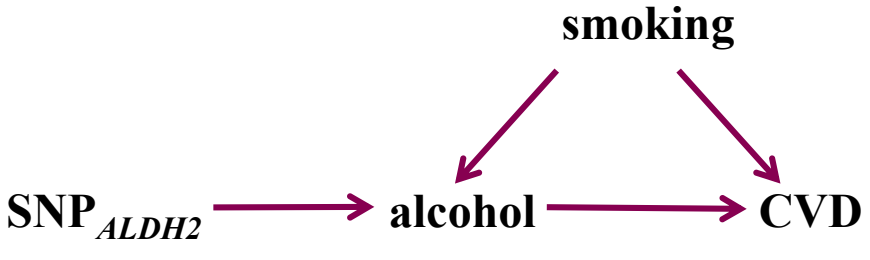
- An instrumental variable (G) should satisfy the following assumptions:
  1. The IV *G* is robustly associated with the exposure of interest *X*
  2. *G* is independent of confounding factors *U* that confound the association of *X* and the outcome *Y*
  3. *G* is independent of outcome *Y* given *X* and confounding factors *U*
     (no pleiotropy)

Table 1 | Three key assumptions that must hold for a Mendelian randomisation study to be valid

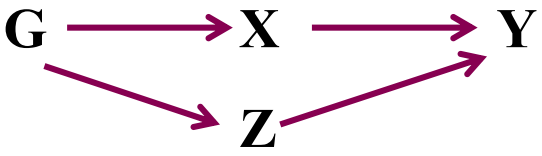
		Tools to assess plausibility	
Assumption	**Description**	**Single sample**	**Two sample**
Relevance assumption	The genetic variants associate with the risk factor of interest	The partial F statistic and partial r squared, or risk difference	Variants are associated with the risk factor in a large genome-wide study
Independence assumption	There are no unmeasured confounders of the associations between genetic variants and outcome	Covariate balance tests and bias component plots. Adjusting for principal components of population stratification	Evidence from large genome-wide association studies on the association of the genetic variants used as instruments with other baseline covariates
Exclusion restriction	The genetic variants affect the outcome only through their effect on the risk factor of interest	Biological knowledge, tests of association of the genetic variants and potential alternative mediating pathways	Evidence from large genome-wide association studies that the genetic variants associate with alternative pathways. MR Egger test for pleiotropy, Cook's distance evaluation of outliers

Neil M Davies et al. BMJ 2018;362:bmj.k601





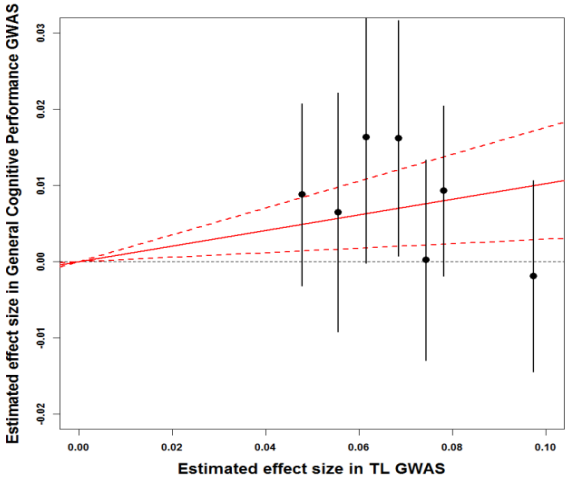
Assumption 3: SNP (G) is independent of outcome (Y) if adjusted for X



Horizontal pleiotropy

Vertical pleiotropy

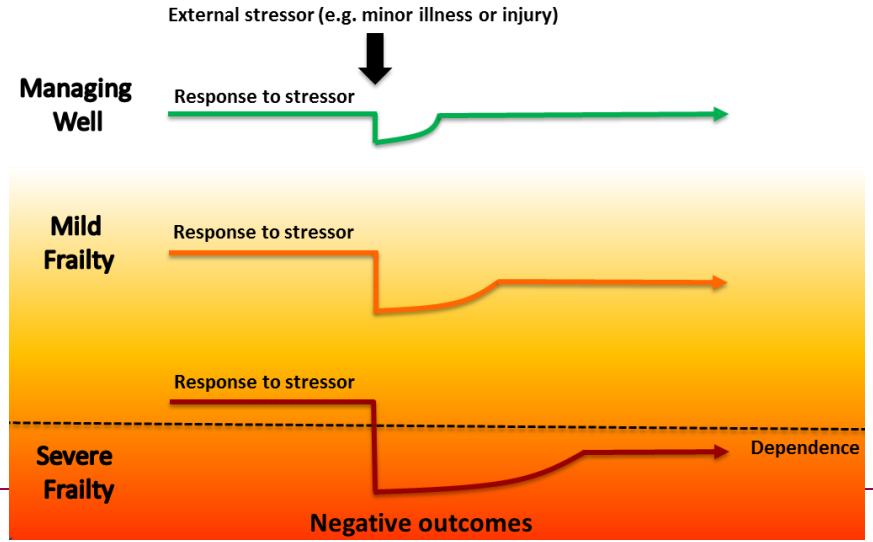
Testing assumption 3



"Analyses were conducted using the inverse variance-weighted, weighted median, MR-PRESSO, MR-Egger, and multivariable MR methods."

What exactly is frailty?

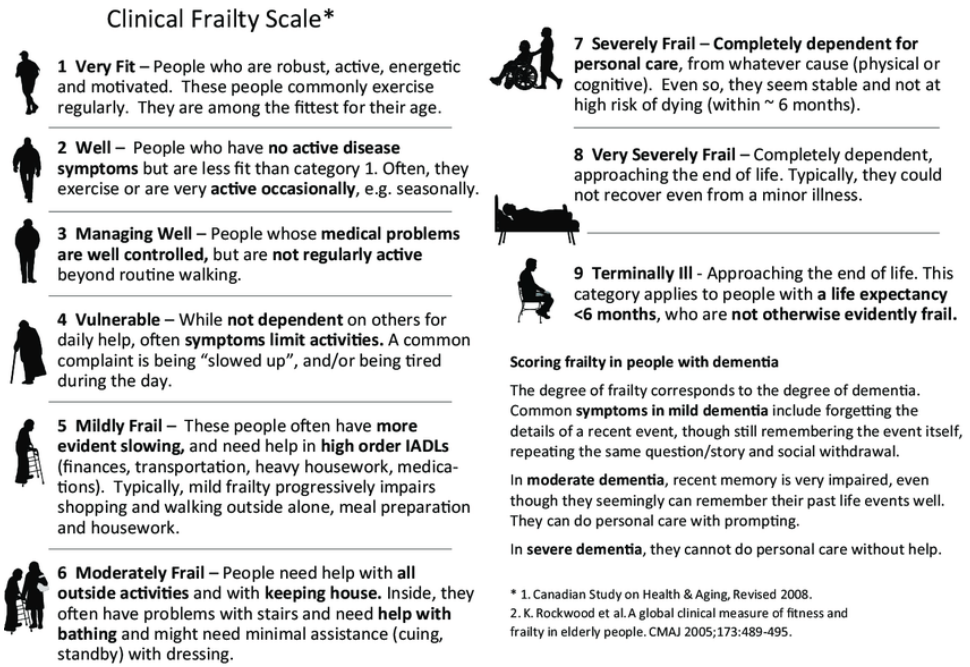
- Conceptually defined as an ”age-associated decline in physiological reserves and function across multiorgan systems making the individual vulnerable to adverse outcomes”
- Strongly predictive of mortality, falls, fractures, disability, hospitalizations, ER visits, morbidity…



https://www2.gov.bc.ca/gov/content/health/practitioner-professional-resources/bc-guidelines/frailty

How to measure frailty?

- ~30 different scales
- Clinically most useful scales
  - Clinical Frailty Scale (CFS)
  - The FRAIL scale
  - Fried phenotypic model



Clinical Frailty Scale*

1 **Very Fit** – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 **Well** – People who have **no active disease symptoms** but are less fit than category 1. Often, they exercise or are very **active occasionally**, e.g. seasonally.

3 **Managing Well** – People whose **medical problems are well controlled,** but are **not regularly active** beyond routine walking.

4 **Vulnerable** – While **not dependent** on others for daily help, often **symptoms limit activities.** A common complaint is being "slowed up", and/or being tired during the day.

5 **Mildly Frail** – These people often have **more evident slowing,** and need help in **high order IADLs** (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 **Moderately Frail** – People need help with **all outside activities** and with **keeping house.** Inside, they often have problems with stairs and need **help with bathing** and might need minimal assistance (cuing, standby) with dressing.

7 **Severely Frail – Completely dependent for personal care**, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 **Very Severely Frail** – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9 **Terminally Ill** - Approaching the end of life. This category applies to people with **a life expectancy <6 months**, who are **not otherwise evidently frail.**

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia**, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia**, they cannot do personal care without help.

* 1. Canadian Study on Health & Aging, Revised 2008.
2. K. Rockwood et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005;173:489-495.

- Most common definitions for research purposes
  - Fried phenotypic model (2001)
  - Rockwood Frailty Index, deficit accumulation model (2002)

Fried phenotypic model (FP)

Weight loss	Unintentional loss of ≥4.5 kg in the past year
Weakness	Hand-grip strength in the lowest 20% quintile adjusted for sex and body mass index
Exhaustion	Poor endurance and energy, self-reported from the Center for Epidemiologic Studies Depression Scale
Slowness	Walking speed under the lowest quintile adjusted for sex and height
Low physical activity level	Lowest quintile of kilocalories of physical activity during the past week, measured by the Minnesota Leisure Activity Scale

Score across the five items:
0=non-frail
1-2=pre-frail
≥3 = frail

Fried et al. 2001. J Gerontol 56 (3): M146–56

Frailty Index (FI)

- Measures the accumulation of deficits: signs, symptoms, diseases, difficulties in functioning, psychosocial well-being etc.
- FI = number of health deficits present/number of health deficits measured
- For example, a person with 8 of 40 deficits considered has an FI of 8/40 =0.2
- Robust and replicative across different cohorts when different items and different numbers of items are used

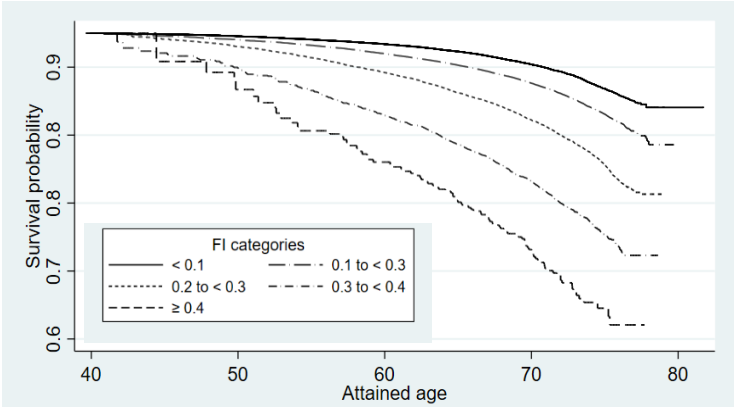
Searle et al. BMC Geriatrics. 2008; 8(1)

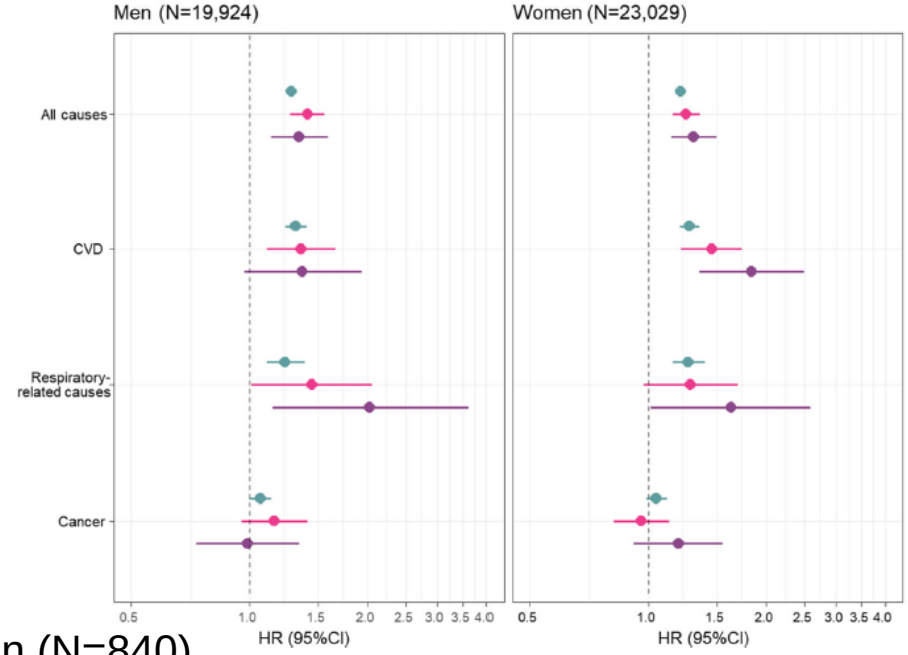
Frailty index – example items from the Swedish SATSA study

Item	Scoring
Hearing status	Perfect=0, Good=0.25, Pretty Good=0.5, Bad=0.75, Deaf or almost deaf=1
Vision status	Perfect=0, Good=0.25, Pretty Good=0.5 , Bad=0.75, Blind or almost blind=1
Health prevents from doing things normally would like to do	No=0, Somewhat=0.5, Yes=1
Self-reported general health	Good=0, Mediocre=0.5, Bad=1
Cancer or leukemia	No=0, Yes=1
Rheumatoid arthritis	No=0, Yes=1
Arthritis	No=0, Yes=1
Chronic bronchitis or emphysema	No=0, Yes=1
Cataracts	No=0, Yes=1
Chest pain	No=0, Yes=1
Circulation problems in arms or legs	No=0, Yes=1
Persistent cough	No=0, Yes=1
Diabetes	No=0, Yes=1
Dizziness	No=0, Yes=1
Gastric ulcer	No=0, Yes=1
Allergies/allergic manifestations	No=0, Yes=1
Asthma	No=0, Yes=1
Shower and bathe	No problem=0, Needs help=0.5, Cannot=1
Get in and out of bed	No problem=0, Needs help=0.5, Cannot=1
Dress and undress	No problem=0, Needs help=0.5, Cannot=1
Self-grooming	No problem=0, Needs help=0.5, Cannot=1
Walking	No problem=0, Needs help=0.5, Cannot=1
Trouble getting to toilet in time	No=0, Yes=1
Manage money	No problems=0, Needs help=0.5, Doesn't do=1
Feeling lonely	Never, almost never, rather seldom=0 Quite often, always, almost always=1
Consider oneself happy and carefree	No=1, Yes=0
Usually feels tired	No=0, Yes=1

FI predicts mortality

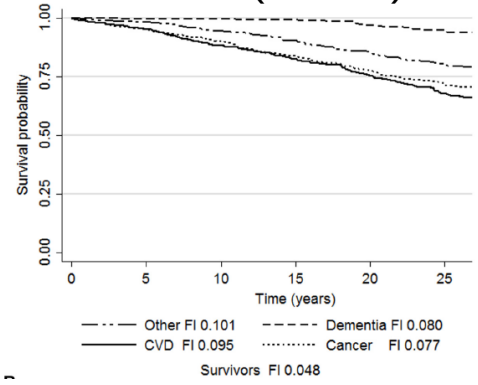
UK biobank



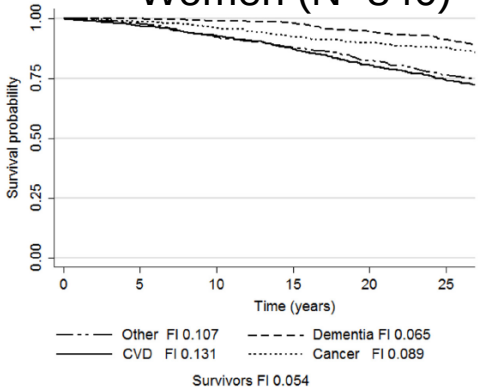


Swedish Twin Registry

Men (N=620)



Women (N=840)



Swedish Adoption/Twin Study of Aging (SATSA)

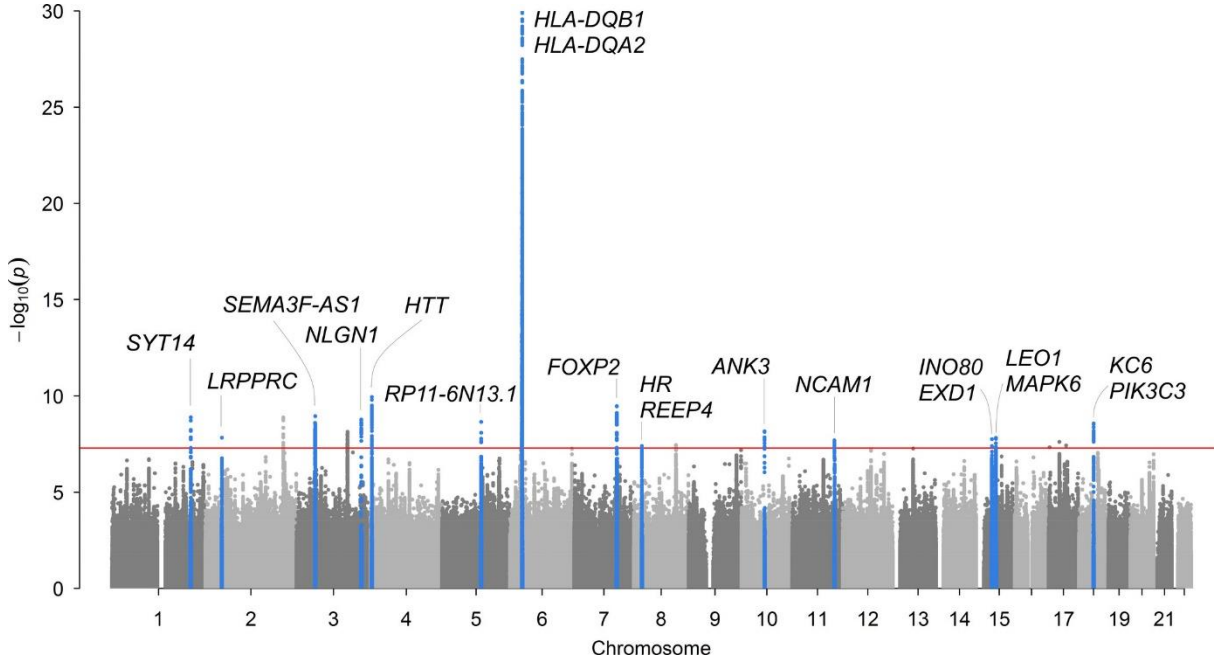
Williams et al. J Gerontol, 2018
Li et al, BMC Med, 2019
Jiang et al. Aging, 2017

Genetic variation and FI

Received: 23 September 2019 | Revised: 14 July 2021 | Accepted: 6 August 2021
DOI: 10.1111/acel.13459
ORIGINAL PAPER
Aging Cell WILEY

A genome-wide association study of the frailty index highlights brain pathways in ageing

Janice L. Atkins[1] | Juulia Jylhävä[2] | Nancy L. Pedersen[2,3] | Patrik K. Magnusson[2] | Yi Lu[2] | Yunzhang Wang[2] | Sara Hägg[2] | David Melzer[1,4] | Dylan M. Williams[2,5] | Luke C. Pilling[1,4]



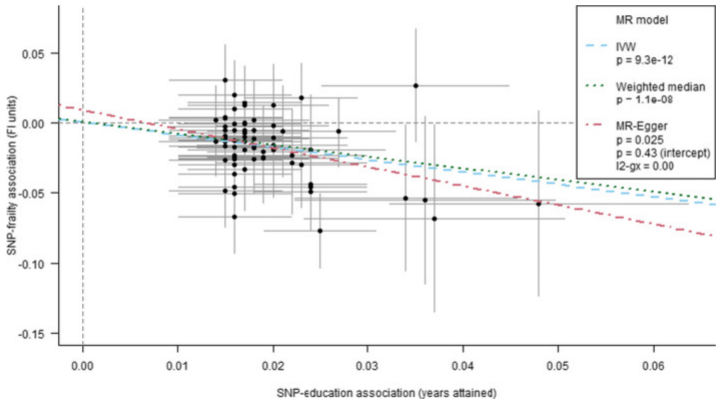
Meta-analysis GWAS of Frailty Index (normalized) in 164,610 UK Biobank participants aged 60–70 of European descent and 10,616 TwinGene participants aged 41–87 years.

Mendelian Randomization: education and FI

Aging Cell Open Access ANATOMICAL SOCIETY

PMC full text:
Aging Cell. 2021 Sep; 20(9): e13459.
Published online 2021 Aug 25. doi: 10.1111/acel.13459

FIGURE 3



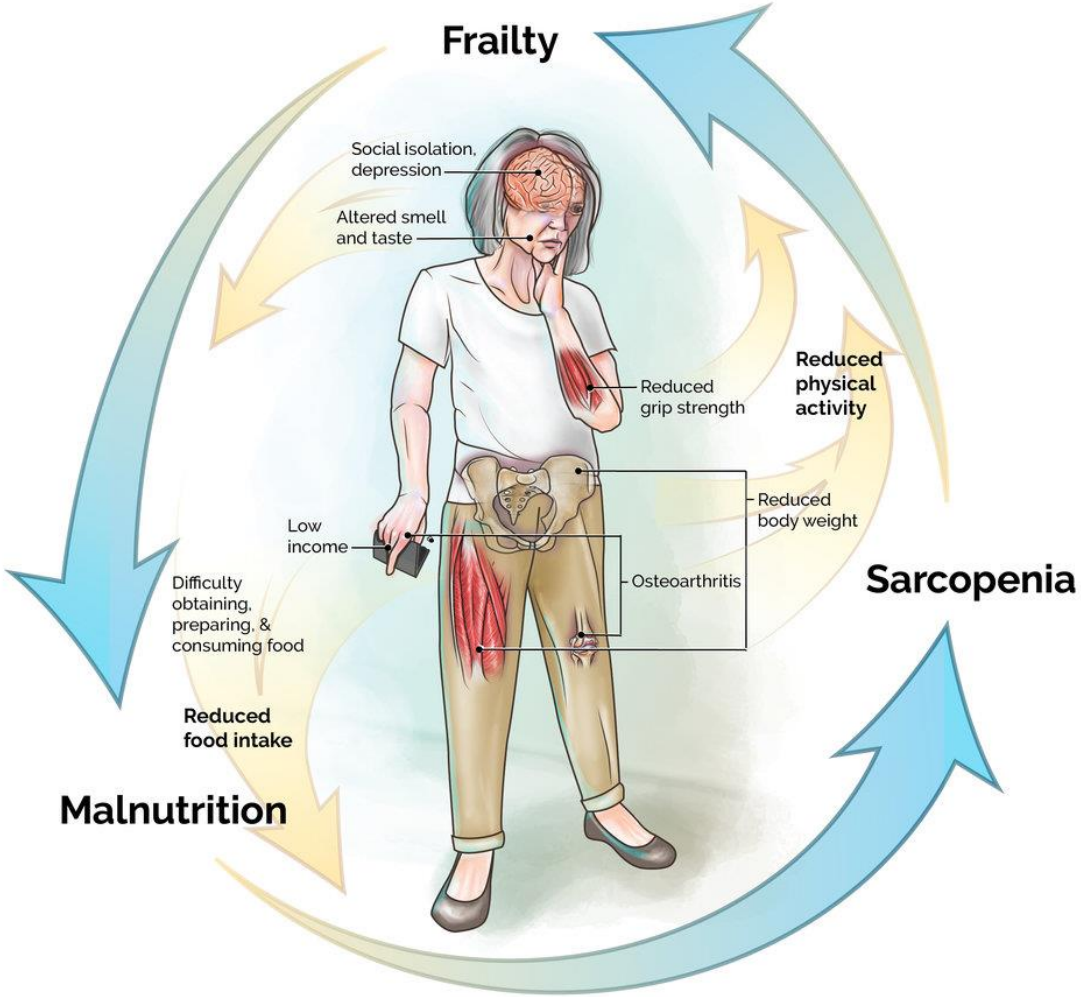
Mendelian randomization estimates for the effect of educational attainment on the frailty index in UK Biobank Points and error bars represent beta estimates and 95% confidence intervals for each SNP-education / SNP-FI association. The trend lines represent different methods for summarizing the estimates from individual SNPS—inverse variance weighting (IVW), weighted median and MR-Egger. The weighted median and MR-Egger estimates are less prone to bias from pleiotropy among the set of variants than IVW, given alternative assumptions hold. The MR-Egger method includes a test of whether the trend's intercept differs from zero, which indicates whether there is an overall imbalance (directional) of pleiotropic effects: such bias was not identified in this education-FI model

SNPs → education → FI

On average, a standard deviation increase (i.e. an additional 3.7 years) in education was predicted to lead to 13.6% lower frailty by the seventh decade of life in UK Biobank participants.

Frailty & nutrition

- Frail individuals are often malnourished
- Frailty is associated with sarcopenia
- Interventions include nutrient supplementations with high protein and energy intake
- RCTs use mixed interventions or are missing



The Journal of Nutrition
Nutritional Epidemiology

Karolinska Institutet

Protein Nutritional Status and Frailty: A Mendelian Randomization Study

Yasutake Tomata,[1,2] Yunzhang Wang,[1] Sara Hägg,[1] and Juulia Jylhävä[1,3]

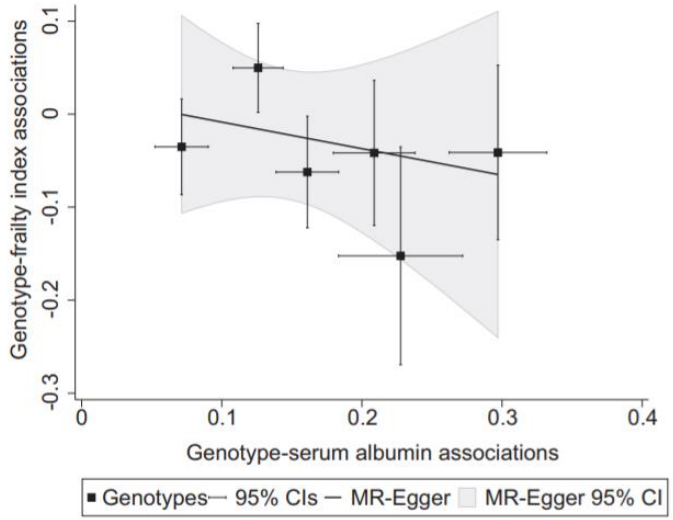


FIGURE 1 Association between genetically predicted serum albumin (g/L) concentrations and frailty index in women ($n = 189,949$): a result of the MR-Egger method.

TABLE 2 MR results of the serum albumin and frailty index by using UK Biobank data[1]

MR method	β	(95% CI)	*P* value
All ($n = 356,432$)[2]			
IVW	−0.023	(−0.141, 0.094)	0.694
Penalized IVW	−0.120	(−0.255, 0.016)	0.083
Weighted median	−0.030	(−0.189, 0.129)	0.712
MR-Egger	−0.015	(−0.330, 0.299)	0.923
MR-Egger (intercept)	−0.001		0.957
Women ($n = 189,949$)[2]			
IVW	−0.172	(−0.336, −0.007)	0.041
Penalized IVW	−0.296	(−0.477, −0.114)	0.001
Weighted median	−0.185	(−0.420, 0.050)	0.122
MR-Egger	−0.286	(−0.691, 0.120)	0.167
MR-Egger (intercept)	0.020		0.546
Men ($n = 166,483$)[2]			
IVW	0.123	(−0.041, 0.287)	0.141
Penalized IVW	0.123	(−0.041, 0.287)	0.141
Weighted median	0.150	(−0.050, 0.349)	0.141
MR-Egger	0.217	(−0.232, 0.667)	0.343
MR-Egger (intercept)	−0.017		0.659

[1] β, coefficient of serum albumin (g/L); IVW, inverse variance weighted method; MR, Mendelian randomization.
[2] Number of participants who were included in the analysis for summary statistics of frailty index.

2022 Jan 11;152(1):269-275. doi: 10.1093/jn/nxab348.

nutrients

MDPI

Karolinska Institutet

Article

Fatty Acids and Frailty: A Mendelian Randomization Study

Yasutake Tomata [1,2,*], Yunzhang Wang [1], Sara Hägg [1] and Juulia Jylhävä [1,3]

Table 3. MR results of the fatty acids and frailty index.

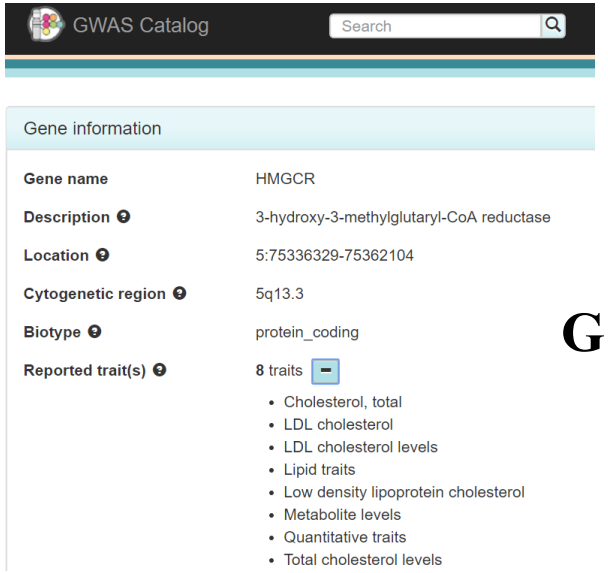
	MR			Multivariate MR [a]		
	β	(95%CI)	p	β	(95%CI)	p
Non-PUFAs						
Saturated fatty acids						
Palmitic acid (16:0)	−0.063	(−0.255, 0.129)	0.518	0.288	(0.128, 0.447)	<0.001
Stearic acid (18:0)	0.178	(0.050, 0.307)	0.007	0.361	(0.155, 0.567)	0.001
Mono-unsaturated fatty acids						
Palmitoleic acid (16:1n-7)	−1.127	(−1.868, −0.387)	0.003	0.026	(−1.083, 1.135)	0.963
Oleic acid (18:1n-9)	−0.304	(−0.458, −0.150)	<0.001	−0.086	(−0.330, 0.158)	0.488
PUFAs						
n-6 PUFAs						
Linoleic acid (18:2n6)	−0.039	(−0.063, −0.016)	0.001	1.075	(−1.549, 3.698)	0.422
Arachidonic acid (20:4n6)	0.039	(0.018, 0.060)	<0.001	−0.266	(−0.937, 0.406)	0.438
n-3 PUFAs						
α-Linolenic acid (18:3n3)	−4.379	(−6.615, −2.143)	<0.001	−44.36	(−186.03, 97.32)	0.539
Eicosapentaenoic acid (20:5n3)	0.722	(0.323, 1.122)	<0.001	−7.865	(−50.28, 34.55)	0.716
Docosapentaenoic acid (22:5n3)	0.849	(0.442, 1.255)	<0.001	23.09	(−51.07, 97.25)	0.542
Docosahexaenoic acid (22:6n3)	−0.088	(−0.390, 0.215)	0.571	4.482	(−5.569, 14.533)	0.382

Abbreviations: MR = Mendelian randomization with inverse variance weighted method using a fixed-effect model; 95%CI = 95% confidence interval; Multivariate MR = Multivariable Mendelian randomization. PUFA = polyunsaturated fatty acids. [a] Multivariate model for non-PUFAs included palmitic acid, stearic acid, palmitoleic acid, and oleic acid as exposure variables. Multivariate model for PUFAs included linoleic acid, arachidonic acid, α-Linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid as exposure variables.

2021 Oct 9;13(10):3539. doi: 10.3390/nu13103539.

MR for estimating the causal effect of drug repurposing on age-related traits

Statins: HMG-CoA reductase inhibitors



Is lifelong lowering of LDL cholesterol protective for the frailty index?

SNP → HMGCR → LDL → FI

Objective: Investigate association between LDL-lowering genetic variants and the frailty index (FI) in the UK Biobank using MR.

GRS (number of SNPs)	Mechanism/drug
Large (274)	All GWAS hits for LDL
Small (50)	Remove pleiotropic SNPs
HMGCR (16)	statins
PCSK9 (34)	alirocumab, evolocumab
NPC1L1 (24)	ezetimibe
APOB (30)	mipomersen
APOC3 (19)	Apolipoprotein C3
LDLR (30)	LDL receptor

EBioMedicine 45 (2019) 487–494

Contents lists available at ScienceDirect

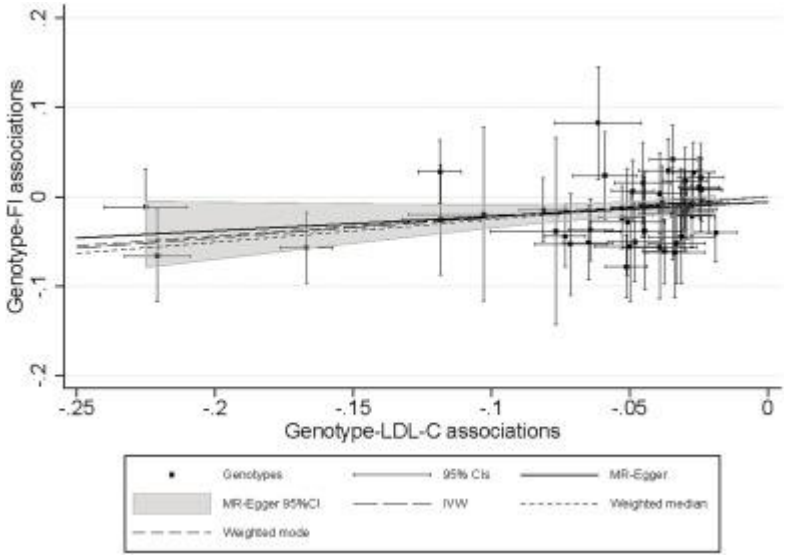
EBioMedicine

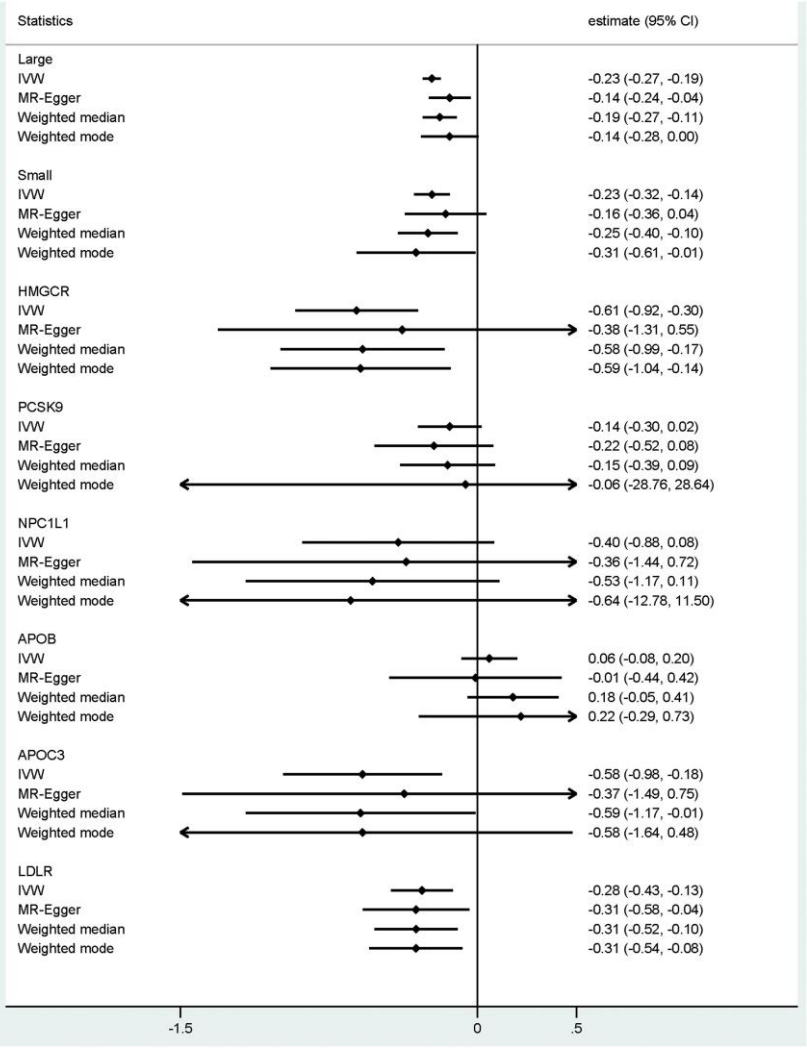
journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Genetically-predicted life-long lowering of low-density lipoprotein cholesterol is associated with decreased frailty: A Mendelian randomization study in UK biobank

Qi Wang [a,b,*], Yunzhang Wang [b], Kelli Lehto [b,c], Nancy L. Pedersen [b,d], Dylan M. Williams [b,e], Sara Hägg [b]





The Strategic Research Area in Epidemiology (SfoEpi) at KI
KI-NIH joint doctoral program
KI Foundations
KI-China Scholarship Council

The Swedish Research Council
The National Institute on Aging (NIA/NIH)
The Swedish Cancer Society
King Gustaf V & Queen Victoria Foundation of Freemason

Thank you!

Aging Epidemiology research group at MEB

Nancy Pedersen
Juulia Jylhävä
Kristina Johnell
Karolina Kauppi
Yunzhang Wang
Xia Li
Ida Karlsson
Ge Bai
Bowen Tang
Chenxi Qin
Thaís Lopes de Oliveira
Malin Ericsson
Adil Supiyev
Jonathan Mak
Maté Szilcz
Jonas Wastesson
KK Kang
Qi Wang
Xueying Qin
Laura Kananen
Peggy Ler
Pierre-Olivier Blotiere
Géric Maura
Yasutake Tomata