

Supplementary Figures

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Fig. S1. Technical flowchart of the analytical pipeline and data flow

This figure provides an overview of the analytical pipeline, explicitly mapping data usage, research questions, and statistical methods at each phase. Detailed descriptions of GWAS data sources, sample sizes, and key study characteristics are summarized in **Table 1**, while the detailed selection process for mediators is presented in **Fig. 3**.

Phase 1 evaluated the associations between genetically predicted birthweight and healthy aging outcomes. In **Phase 1a**, UVMR was applied to estimate the associations between genetically predicted birthweight and two composite healthy aging phenotypes (aging-GIP and mvAge), as well as six individual aging phenotypes. In **Phase 1b**, MVMR was used to assess whether the identified associations were independent of later-life adiposity, by adjusting for childhood BMI, adulthood BMI, or WC. In **Phase 1c**, exploratory analyses further examined the origins of these associations using fetal- and maternal-specific genetic instruments for birthweight via UVMR and MVMR. In **Phase 1d**, replication analyses repeated the primary and exploratory analyses using a distinct birthweight GWAS to assess robustness.

In **Phase 2**, utilizing the two-step MR framework, we estimated the effect of genetically predicted birthweight on each mediator (**Step 1**) and the effect of each mediator on healthy aging outcomes, adjusting for birthweight (**Step 2**). The individual mediation proportion was calculated using the Delta method.

This figure should be interpreted in conjunction with **Fig. 1**, **Fig. 3**, and **Fig. S2**.

Full methodological details are described in the **Methods** section.

Abbreviations: 2hGlu, 2h-glucose post-challenge; aging-GIP, genetically independent phenotype of aging; ALT, alanine aminotransferase; ApoB, apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; CHD, coronary heart disease; GWAS, genome-wide association study; IV, instrumental variables; IVW, inverse-variance weighted; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual sum and outlier; mvAge, multivariate aging-related genetic factor; MV-IVW, multivariable inverse-variance weighted; MVMR, multivariable Mendelian randomization; nonHDL-C, non-high-density lipoprotein cholesterol; PhenoAgeDecel, phenotypic age deceleration; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; UACR, urine albumin-to-creatinine ratio; UVMR, univariable Mendelian randomization; WC, waist circumference.

Evaluation of the association between genetically predicted birthweight and healthy aging phenotypes

Phase 1a. UVMR establishes the associations between genetically predicted birthweight and healthy aging phenotypes

- **Exposure:** birthweight ($N=298,142$)
- **Outcome:** aging-GIP, mvAge, healthspan, resilience, parental lifespan, self-rated health, PhenoAgeDecel, longevity ($N=34,710$ to $1,958,774$)
- **Statistical approach:** UVMR (IVW, Weighted median, Weighted mode, Simple mode, MR-Egger, MR-PRESSO, MRlap)



Phase 1b. MVMR tests if the identified significant associations are independent of later-life obesity

- **Exposure:** birthweight ($N=298,142$)
- **Covariate:** childhood BMI, adulthood BMI, or adulthood WC ($N=39,620$ to $681,275$)
- **Outcome:** significant aging phenotypes from Phase 1a
- **Statistical approach:** MVMR (MV-IVW, MVMR-Egger, MVMR-Median, MVMR-Lasso)



Phase 1c. Exploratory analysis

- **Exposure:** fetal-effect birthweight, fetal-only birthweight, maternal-effect birthweight, maternal-only birthweight instruments ($N=406,063$)
- **Covariate:** childhood BMI, adulthood BMI, or adulthood WC ($N=39,620$ to $681,275$)
- **Outcome:** aging-GIP, mvAge, healthspan, resilience, parental lifespan, self-rated health, PhenoAgeDecel, longevity ($N=34,710$ to $1,958,774$)
- **Statistical approach:** UMVR and MVMR



Phase 1d. Replication analysis

- **Exposure:** birthweight GWAS from *Juliusdottir T, et al.* ($N=104,920$ to $423,683$)
- **Covariate:** childhood BMI, adulthood BMI, or adulthood WC ($N=39,620$ to $681,275$)
- **Outcome:** aging-GIP, mvAge, healthspan, resilience, parental lifespan ($N=154,478$ to $1,958,774$)
- **Statistical approach:** UMVR and MVMR

Identification of the qualified mediators between birthweight and healthy aging

Phase 2 Step 1. UVMR estimates the effect of birthweight on each mediator (β_1)

- **Exposure:** birthweight ($N=298,142$)
- **Outcome:** each mediator (educational attainment, household income, occupational attainment, leisure screen time, cheese intake, age at first sex, age at first live birth, grip strength, SBP, fasting glucose, 2hGlu, total cholesterol, triglycerides, LDL-C, nonHDL-C, ApoB, UACR, ALT, SHBG, type 2 diabetes, hypertension, CHD, CAD, MI, coronary atherosclerosis, angina pectoris) ($N=24,925$ to $1,812,017$)
- **Statistical approach:** UVMR (IVW, Weighted median, Weighted mode, Simple mode, MR-Egger, MR-PRESSO, MRlap)



Phase 2 Step 2. MVMR estimates the effect of each mediator on healthy aging with adjustment for birthweight (β_2)

- **Exposure:** each mediator ($N=24,925$ to $1,812,017$)
- **Covariate:** birthweight ($N=298,142$)
- **Outcome:** aging-GIP, mvAge ($N=154,478$ to $1,958,774$)
- **Statistical approach:** MVMR (MV-IVW, MVMR-Egger, MVMR-Median, MVMR-Lasso)



Calculate the mediation proportion

- **Exposure:** birthweight ($N=298,142$)
- **Mediator:** each mediator ($N=24,925$ to $1,812,017$)
- **Outcome:** aging-GIP, mvAge ($N=154,478$ to $1,958,774$)
- **Statistical approach:** Delta method

Fig. S2. Genetic instrument construction and outcome datasets used in the association between birthweight and healthy aging

This figure complements **Fig. S1** by providing instrument-level details underlying the Phase 1 UVMR analyses, including genetic instrument selection, harmonization, and outcome datasets.

For the primary analysis, genetic instruments for birthweight were obtained from a large-scale GWAS meta-analysis of 298,142 individuals of European ancestry (Warrington NM *et al.*). Independent SNPs were selected using stringent linkage disequilibrium (LD) clumping ($r^2 < 0.001$ within a 10,000 kb window) based on the 1000 Genomes European reference panel, yielding 124 IVs for birthweight. For exploratory analyses, fetal- and maternal-specific genetic instruments were derived based on structural equation modeling classifications from the primary GWAS (Warrington NM *et al.*). We derived: (1) 51 fetal-only IVs and 77 fetal-effect IVs with adjustment for maternal genotype to represent the fetal genetic effects on birthweight; (2) 29 maternal-only IVs and 54 maternal-effect IVs with adjustment for fetal genotype to capture the maternal genetic effects on birthweight. For replication analyses, genetic instruments were extracted from an independent birthweight GWAS (Juliusdottir T *et al.*). We extracted all 196 SNPs as overall birthweight instruments and SNPs corresponding to fetal-only (clusters 1-3), fetal-effect (clusters 1-3, 5-8), maternal-only (cluster 4), and maternal-effect (clusters 4-8) groups as originally classified. Instruments were harmonized with outcome GWAS, with strand alignment and removal of ambiguous palindromic SNPs where necessary.

Abbreviations: aging-GIP, genetically independent phenotype of aging; GWAS, genome-wide association study; IVs, instrumental variables; LD, linkage disequilibrium; mvAge, multivariate aging-related genetic factor; SNP, single-nucleotide polymorphism.

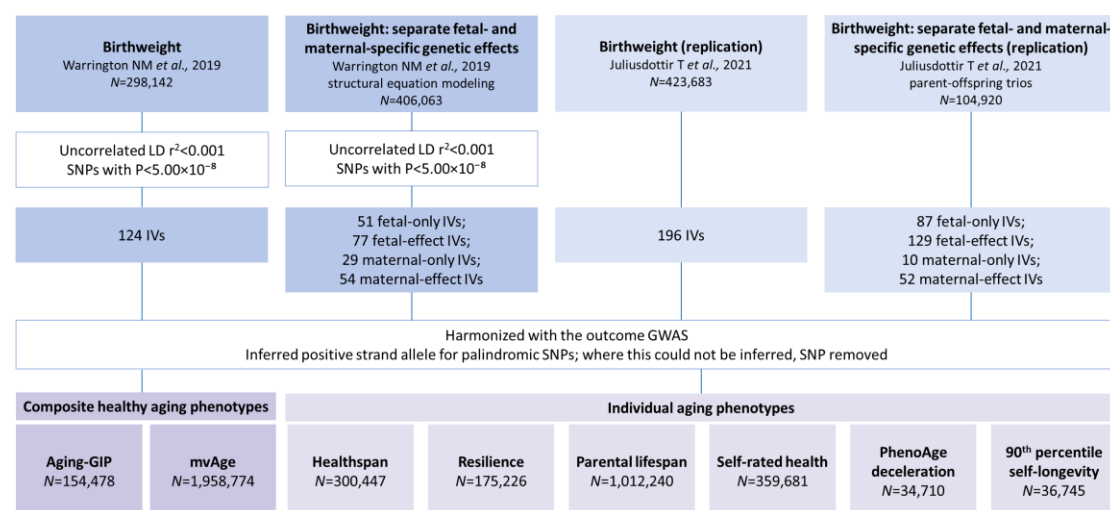
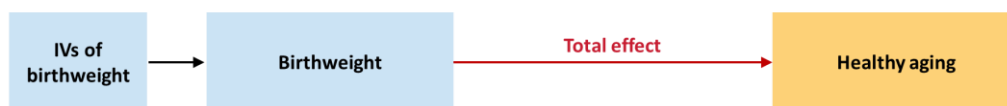


Fig. S3. DAG illustrating the causal hypotheses and MR framework

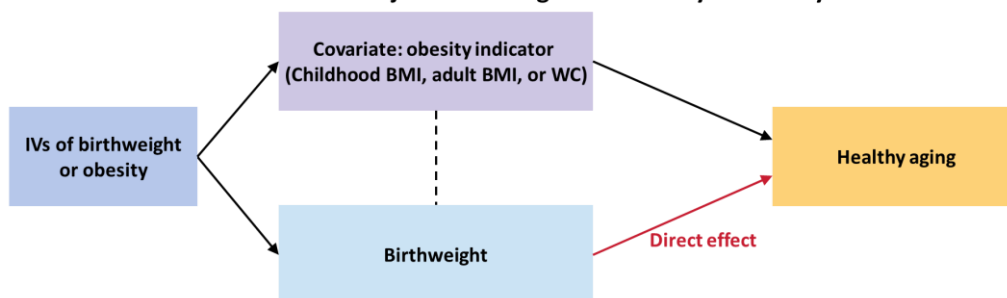
This DAG represents the conceptual causal hypotheses and the corresponding MR frameworks utilized to estimate the total, direct, and mediating effects of birthweight on healthy aging. The red arrows denote the specific causal estimates targeted in each analytical phase.

a, UVMR framework. This model estimates the total causal effect of birthweight on healthy aging. **b**, MVMR framework. In this model, genetic instruments were the combination of SNPs, which were genome-wide significant in either the GWAS of birthweight or the GWAS of a single obesity indicator (childhood BMI, adulthood BMI, or WC) and were independent of each other. The line between birthweight and obesity indicator is left dashed as no assumptions are made about this relationship in the estimation of the direct effect. This approach estimates the direct effect of birthweight, conditional on genetic liability to obesity. **c**, Two-step MR framework. Step 1 estimates the effect of birthweight on each mediator (β_1). Step 2 estimates the direct effect of each mediator on healthy aging after adjusting for birthweight (β_2). The mediation effect is quantified as the product of coefficients ($\beta_1 \times \beta_2$). Abbreviations: BMI, body mass index; DAG, directed acyclic graph; GWAS, genome-wide association study; IV, instrumental variable; MR, Mendelian randomization; MVMR, multivariable Mendelian randomization; SNP, single nucleotide polymorphism; UVMR, univariable Mendelian randomization; WC, waist circumference.

a. DAG in Phase 1 UVMR model



b. DAG in Phase 1 MVMR with adjustment for genetic liability to obesity



c. DAG in Phase 2 mediation analysis

