Title:

Association between birth weight and refractive error in adulthood: a Mendelian randomization study.

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Keywords:

Refractive error; Birth weight; Mendelian randomisation; UK Biobank

Conflict of Interest:

The authors declare that they have no conflict of interest.

Acknowledgements:

This research has been conducted using the UK Biobank Resource (applications #17351 and #17615). Data analysis was carried out using the RAVEN computing cluster, maintained by the ARCCA group at Cardiff University ARCCA and the BLUE CRYSTAL3 computing cluster maintained by the HPC group at the University of Bristol. This research was specifically funded by NIHR Senior Research Fellowship award SRF-2015-08-005 and the Global Education Program of the Russian Federation government. UK Biobank was established by the Wellcome Trust; the UK Medical Research Council; the Department for Health (London, UK); Scottish Government (Edinburgh, UK); and the Northwest Regional Development Agency (Warrington, UK). It also received funding from the Welsh Assembly Government (Cardiff, UK); the British Heart Foundation; and Diabetes UK. Collection of eye and vision data was supported by The Department for Health through an award made by the NIHR to the Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust, and UCL Institute of Ophthalmology, London, United Kingdom (grant no. BRC2_009). Additional support was provided by The Special Trustees of Moorfields Eye Hospital, London, United Kingdom (grant no. ST 12 09).

Synopsis

A Mendelian randomisation analysis to estimate the causal effect of birth weight on refractive error in adulthood suggested that each 1 standard deviation reduction in birth weight caused a -0.28 D more negative refractive error.

Abstract

Background. Pathological myopia is one of the leading causes of blindness globally. Lower birth weight (BW) within the normal range has been reported to increase the risk of myopia, although findings conflict. We sought to estimate the causal effect of BW on refractive error using Mendelian randomization, under the assumption of a linear relationship.

Methods. Genetic variants associated with BW were identified from meta-analysis of a genome-wide association study (GWAS) for self-reported BW in 162,039 UK Biobank participants and a published EGG consortium GWAS (n=26,836). We performed a 1-sample Mendelian randomization (MR) analysis in 39,658 unrelated, adult UK Biobank participants (independent of the GWAS sample) using an allele score for BW as instrumental variable. A 2-sample MR sensitivity analysis and conventional ordinary least squares (OLS) regression analyses were also undertaken.

Results. In OLS analysis, BW showed a small, positive association with refractive error: +0.04D per standard deviation (SD) increase in BW (95% CI: 0.02 to 0.07; p=0.002). The 1-sample MR-estimated causal effect of BW on refractive error was higher, at +0.28D per SD increase in BW (95% CI: 0.05 to 0.52, p=0.02). A 2-sample MR analysis provided similar causal effect estimates, with minimal evidence of directional pleiotropy.

Conclusions. Our study suggests lower birth weight within the normal range is causally associated with a more myopic refractive error. However, the impact of the causal effect was modest (range 1.00D covering approximately 95% of the population).

Introduction

Myopia is a common eye disorder that increases the risk of maculopathy, glaucoma, cataract, and retinal detachment [1,2]. Despite numerous epidemiologic and genetic studies, the risk factors and mechanisms underlying myopia development are still not fully understood [3-5].

Myopia is more common in children with extremely low birth weight (BW), either with or without retinopathy of prematurity [6,7], although an increased prevalence of hyperopia and astigmatism has also been reported [8,9]. The ocular structure primarily responsible for causing myopia in individuals with extremely low BW is unclear, since both corneal curvature and axial length have been reported to differ in preterm vs. full-term-born infants, and there is evidence suggesting some of these differences may resolve during childhood [10-12]. However, few epidemiological studies have assessed the relationship between refractive error and BW within the normal range, either for adult or child samples. In a cohort of infants from Singapore [13] a trend towards a negative association of BW and refraction was observed, i.e. a more hyperopic refractive error with lower BW. In contrast, a study of 12-13 year-old children from Northern Ireland [14] reported a positive association. In both studies, statistical support for the association was weak. No association between BW and refractive error was found in 12 to 15 year-old adolescent participants in the US National Health and Nutrition Examination Survey (NHANES), although BW was associated with corneal curvature [11]. In adult participants from the population-based 1958 British Birth Cohort [15], myopia was associated with lower BW for gestational age (OR=0.90, P<0.05) and in the population-based Gutenberg Health Study [16], refractive error was -0.017 D (95% CI -0.011 to -0.023; P<0.001) more myopic per standard deviation reduction in BW.

Previous investigations of the relationship between BW and refractive error have been restricted to observational study designs. Observational studies are susceptible to confounding, and therefore offer very limited potential for drawing causal inferences [17]. The aim of the current study was to test for a *causal* relationship between BW within the normal range and refractive error in adulthood. For this purpose, we used a Mendelian randomization (MR) study design. In MR, genetic variants associated with an exposure (risk factor) are used as 'instrumental variables' to provide effect estimates free from bias due to reverse causality and that are less prone to bias from unmeasured confounders than conventional observation studies [8,18]. The MR approach has been likened to a randomized controlled trial [19]. Three assumptions are necessary in order for a specific genetic variant to be used as an instrumental variable in an MR analysis (Figure 1)*:* i) *the genetic variant must be associated with the level of the exposure variable;* ii) *the genetic variant must not be*

associated with any confounders of the exposure-outcome relationship; and, iii) *the genetic variant must be associated with the outcome only via the exposure* [20,21]. Newer variations of MR allow the relaxation of assumptions (ii) and (iii) to some extent [22].

Methods

UK Biobank

UK Biobank is a longitudinal study of approximately 500,000 participants aged 37-73 years, recruited between 2006 and 2010 [23]. Ethical approval was obtained from the National Health Research Ethics Service (Ref 11/NW/0382) and all participants provided written informed consent. Genotype data from the UK Biobank July 2017 full release were available for 488,377 participants. Data for BW were available for 279, 971 participants, who recalled their BW at the baseline and/or follow-up assessments. BW data were Z-transformed: (BW value – mean BW)/ standard deviation (SD) of BW. A flow diagram outlining the selection of UK Biobank participants for the GWAS and MR analyses is presented in Figure 2. The validity of self-reported BW was previously assessed by Tyrrell et al. [24], who reported it to be associated with non-singleton pregnancy, female sex, maternal smoking at the time of pregnancy, earlier year of birth, and socioeconomic status. All of these associations were in the expected directions.

Approximately 23% of UK Biobank participants underwent an ophthalmic assessment, which included non-cycloplegic autorefraction/keratometry (Tomey RC5000; Tomey Gmbh Europe, Germany). Up to 10 repeat measurements were taken. Mean Spherical Equivalent (MSE) refractive error was calculated over repeat measurements for each eye separately, after excluding readings flagged as being unreliable: MSE = Sphere + (0.5*Cylinder). The average MSE (avMSE) of the 2 eyes was used as the participant's refractive error in the statistical analyses.

A set of well-imputed genetic variants (with IMPUTE4 [25] INFO metric > 0.9, minor allele frequency (MAF) > 0.005, missing rate $\leq$ 0.01, and an 'rs' variant ID prefix that were LD-pruned using the --indep-pairwise 50 5 0.1 command in in PLINK 2.0 [26]) was used to create a genetic relationship matrix (GRM). From the set of n=409,728 White British ancestry individuals identified by Bycroft et al. [25] a group of (essentially) unrelated individuals was selected using the PLINK 1.9 [26] --rel-cutoff 0.025 command in conjunction with the GRM. A total of 338,256 unrelated White British ancestry individuals was identified. For these participants, the mean and SD of the each of the first 20 Principal Components (PCs) of genetic data were calculated (using the PCs obtained by Bycroft et al.

[25]). Individuals from the full cohort who were within ±10 SD of the mean for each of the first 20 PCs were retained (n=446,678). We excluded a further 1,821 participants with self-reported non-white ethnicity and those with autosomal heterozygosity more than 4 SD from the mean. Individuals with valid autorefraction data, no history of eye surgery and who had not withdrawn consent were utilized as the sample for a GWAS for refractive error (n=95,504; see below). Following the selection criteria adopted by Horikoshi et al. [27] from the remaining 347,708 participants we excluded participants who had withdrawn consent, those with BW outside the range of 2.5-4.5kg, those from multiple births (e.g. twins) and those who reported a difference in BW > 0.5kg between the baseline and follow-up assessments. This resulted in a total sample of 162,039 participants for inclusion in a GWAS for BW (Figure 2).

For the GWAS for refractive error we excluded participants from downstream analyses if they: (i) self-reported a history of laser refractive surgery, cataract or corneal graft surgery, or any type of eye surgery within the last 4 weeks before the assessment; (ii) self-reported any eye trauma that led to sight loss; (iii) self-reported cataract or retinal detachment; (iv) had a hospital record of ocular surgery. This resulted in 95,692 participants for further analysis.

A subgroup of unrelated UK Biobank participants (n=39,658) with both autorefraction and BW data available was used for the 1-sample MR analysis and the OLS analysis (Figure 2).

EGG consortium GWAS meta-analysis for birth weight

Summary statistics for a GWAS meta-analysis for BW were downloaded from the Early Growth Genetics (EGG) consortium website www.egg-consortium.org [28]. The EGG consortium meta-analysis comprised of 18 European population-based studies (n=26,836).

CREAM consortium GWAS meta-analysis for refractive error

Summary statistics for a GWAS meta-analysis for refractive error were provided by the CREAM consortium. The CREAM meta-analysis was based on 29 studies of European ancestry (n=44,192 in total), most of which were population based. Spherical equivalent averaged between the two eyes was used as the refractive error phenotype. The 29 CREAM studies did not adjust for BW when performing the GWAS analyses for refractive error.

Ordinary Least Squares (OLS) Regression

To examine the observational association between BW and refractive error we performed a linear regression analysis in the sample of UK Biobank participants with information available for both refractive error and BW (n=39,658), adjusting for age and sex. BW was coded as a Z-score, i.e. 1 SD of BW was used as the unit of measurement.

GWAS for birth weight and for refractive error in UK Biobank participants

Single marker association tests for BW (coded as a Z-score) and for refractive error (coded in Dioptres) were carried out genome-wide in n=162,039 and n=95,504 individuals, respectively, from UK Biobank, using BOLT-LMM [29]. We included 10.4 million genetic markers present on the HRC reference panel [25] with MAF $\geq$0.05 and IMPUTE4 INFO metric >0.9 and per-marker missing rate <0.015 and per-individual missing genotype rate <0.025. Age, gender, genotyping array (coded as 0 or 1 for the UK BiLEVE or UK Biobank Axiom, respectively) and the first 10 PCs were included as covariates. The genetic relationship matrix for the BOLT-LMM analysis was created as described above. The GWAS summary statistics were filtered to remove markers with a p<0.01 for a test of Hardy-Weinberg equilibrium.

Meta-analysis of GWAS summary statistics

Two meta-analyses were conducted. Firstly, a meta-analysis of the two sets of BW GWAS summary statistics (n=162,039 individuals from UK Biobank and n=26,836 individuals from the EGG Consortium) and secondly a meta-analysis of the two sets of refractive error GWAS summary statistics (n=95,504 individuals from UK Biobank and n=44,192 individuals from the CREAM Consortium). We used a fixed effects, inverse variance-weighted meta-analysis model in METAL [30].

An allele score for birth weight

The BW GWAS meta-analysis identified 75 lead SNPs associated with BW at P<5.0e-08 that were separated by at least 500 kb and that had pairwise linkage disequilibrium (LD) $r^2 < 0.2$. Two of these SNPs were not present in the CREAM refractive error GWAS summary statistics, leaving 73 SNPs for deriving an allele score. These allele scores were calculated for UK Biobank individuals using the PLINK 1.9 '--score' command, with weighting based on the beta-coefficient quantifying the degree of association with BW.

The variance in BW explained by the allele score was assessed in the sample of unrelated UK Biobank participants with information available for both refractive error and BW (n=39,658; note that there was no overlap between this sample of 39,658 participants and the UK Biobank sample

used for the BW GWAS meta-analysis; see Figure 2). A baseline regression model was fit, with BW (Z-scores) as the dependent variable and age, sex, genotyping array (UK BiLEVE array or UK Biobank Axiom array) and the first 10 PCs as predictors. A full model was fit as for the baseline model, with allele score included as an additional predictor. The variance in BW explained by the allele score was calculated as the increase in the adjusted R^2 of the full vs. baseline model.

Mendelian randomization

We performed a 1-sample Mendelian randomization analysis in unrelated UK Biobank participants using the allele score for BW as the instrumental variable and a Limited Information Maximum Likelihood (LIML) model (ivmodel R package). F-statistics from the first stage of a two-step least squares (2SLS) analysis (ivreg function from R package AER) were used to assess the strength of the instrumental variable [31].

As an allele score-based MR analysis can be biased by unbalanced (directional) pleiotropy, we performed MR-Egger and weighted-median (2-sample) MR sensitivity analyses [20,21]. The same set of 73 genetic variants used in the allele score analyses described above were used as instrumental variables. For the analysis results presented in Table 1, regression coefficients and standard errors for the association of these 73 genetic variants with refractive error were obtained from a meta-analysis of GWAS for refractive error in UK Biobank participants and the CREAM Consortium GWAS.

Sample overlap

The EGG and CREAM genetic consortia samples were partially overlapping (e.g. the 1958 British Birth Cohort, EPIC, ERF and ORCADES studies were common between the two datasets). We estimated the maximum possible overlap, was 4,813 individuals, i.e. for example, if the number of ORCADES participants in EGG was X and the number of ORCADES participants in CREAM was Y, then the maximum overlap would be min(X,Y). It was also possible that some participants in the UK-based EGG or UK-based CREAM studies were also enrolled in the UK Biobank study. For the MR analysis using UK Biobank + EGG summary statistics for stage 1 and CREAM + the separate group of UK Biobank for stage 2, we estimated the maximum possible overlap to be 11,685 individuals, or 8% of the stage 2 sample. Thus, the potential for weak instrument bias due to this overlap was limited [32].

Results

GWAS meta-analysis and allele score for birth weight

The UK Biobank sample selected for the GWAS for self-reported BW was restricted to individuals with self-reported BW within the range 2.5-4.5 kg. A comparison of participants included vs. excluded based on this criterion identified that the excluded participants were more often older, male, and from more socially deprived areas (all p<1.0e-73; Supplementary Tables S1-S3; Supplementary Figure S1). The excluded participants were also less likely to have undergone the ophthalmic assessment, and had a more hyperopic refractive error: the median (IQR) refractive error was +0.09 D (-1.28 to 1.05) vs. +0.23 D (-1.12 to 1.27) for included vs. excluded participants (p=7.3e-49). The final sample size for the GWAS was 162,309 (Figure 2).

The GWAS identified 63 SNPs independently associated with self-reported BW at genome-wide significance, p<5.0e-08 (Supplementary Table S4). A meta-analysis of the UK Biobank (n=162,309) and EGG consortium (n=26,836) BW GWAS summary statistics identified 75 genetic variants independently associated with BW (Supplementary Table S5). Two of these SNPs (rs1530624 and rs1058026) were not available in the CREAM meta-analysis for refractive error and hence were excluded. The remaining 73 genetic variants were used to calculate an allele score for BW. The allele score explained 1.2% of the variance in BW in an independent sample of 39,658 UK Biobank participants (p<2.2e-16).

Observational and causal relationship between birth weight and refractive error

The observational association between refractive error and BW was assessed using OLS regression in 39,658 unrelated UK Biobank participants of European ancestry, after adjusting for age and sex. Each 1 SD increase in BW was associated with a +0.04 D increase in refractive error (95% CI 0.02 to 0.07, p=0.002), suggesting that lower BW was a risk factor for myopia and higher BW a risk factor for hypermetropia.

In the same sample of 39,658 UK Biobank participants with information available for both BW and refractive error, a 1-sample Mendelian randomization analysis using the allele score for BW as an instrumental variable estimated the causal effect of BW on refractive error as +0.28 D per 1 SD increase in BW (95% CI 0.05 to 0.52, p=0.02). The F-statistic from the first stage regression of a 2SLS analysis was 496.74, confirming that the allele score was not a 'weak instrument' [33].

A 2-sample MR sensitivity analysis was carried out using the largest datasets available: meta-analysed data for BW in 188,039 participants from UK Biobank and the EGG consortium; and meta-

analysed data for refractive error in 139,696 participants from UK Biobank and the CREAM consortium. The 73 SNPs independently associated with BW were used as instrumental variables. An MR-Egger sensitivity analysis, designed to test for directional pleiotropy, yielded an MR-Egger intercept estimate of 0.00 D, suggesting an absence of directional pleiotropy (Table 1; Figure 3). A weighted median-based MR causal effect estimate, which remains valid even a proportion of the genetic variants do not meet the assumptions necessary for a valid instrumental variable, yielded an MR effect estimate similar to that from the allele score-based MR analysis (Table 1): +0.18 D per 1 SD increase in BW (95% CI 0.02 to 0.35, p=0.03).

Discussion

In this Mendelian randomization study, we estimated the causal effect of BW within the normal range on refractive error as approximately +0.28 D per 1 SD increase in BW. The MR-based estimate of the causal effect of BW was higher than the observational analysis-based estimate (Durbin-Wu-Hausman endogeneity test p=0.04) suggesting bias in the observational analysis or the presence of gene-environment interaction.

The likely reason for the difference between the causal effect estimate from the MR analysis and that estimated from the observational estimate is confounding (typically, observational estimates are more severely affected by confounding bias than are MR estimates). Such confounding may arise from the complex interrelationships between BW and other traits, both of the mother and the proband (Figure 4). For example, an inverted U-shaped relationship between maternal age and BW has been reported [34], and maternal age is negatively correlated with refractive error of the child [33]. In addition, birth order is associated with BW [35] and refractive error [36] (specifically, first-born offspring have a higher risk of myopia development than their siblings). Furthermore, higher maternal education is associated with a reduced risk of low BW in offspring [37]. Similarly, a correlation between refractive error in children and their mother's level of educational attainment has been reported [38], and the level of maternal education is related to maternal age.

Strengths of this study were that the allele score used as an instrumental variable in the primary MR analysis was strongly associated with the exposure (F=496.74), and that sensitivity analyses were carried out to check the robustness of the findings to departures from the assumptions required in MR. We integrated GWAS summary statistics from existing large GWAS datasets and UK Biobank, which provided sufficient statistical power to detect and estimate the causal effect precisely.

One limitation of our study is that it was restricted to participants of European ancestry, while the prevalence of myopia is greater in East and South-East Asian countries compared to Europe. Furthermore, the UK Biobank cohort is not fully representative of the UK population [23] (a phenomenon potentially exacerbated by our exclusion of participants with self-reported BW outside the 2.5 - 4.5 kg range). The causal effect estimate and the observational association between BW and refractive error may differ across population groups or geographic locations as a result of genetic or environmental influences. Secondly, despite the evidence against departure from the MR assumptions, the possibility of pleiotropic effects cannot be ruled out since the biological role of the vast majority of the genetic variants we used as instrumental variables is unknown. For instance, it is plausible that a genetic variant identified in a GWAS for BW has a causal pathway that acts via the mother's level of educational attainment, i.e. a variant associated with higher educational attainment would be expected to show a positive association with BW. Without further knowledge of either the maternal genotype or maternal phenotype of the participants in an MR study, it is not possible to account for phenotypic effects mediated by the maternal genotype and environment separately from those of the alleles inherited by probands [39-41]. Thirdly, a further limitation related to that already discussed above, is that Mendelian randomisation does not elucidate the causal pathway linking an exposure to an outcome. Indeed such causal pathways could potentially be unanticipated and indirect; for example, the relationship between birth order and myopia has been suggested to be mediated at least in part by greater parental investment in the education of first-born children, coupled with a causal relationship between education and myopia [42,43]. Fourthly, the data analysed in the GWAS for BW were obtained by self-report, which may be inaccurate. Moreover, we excluded participants with a self-reported BW outside the normal range (and the participants excluded based on this criterion were not a random sample of the full UK Biobank cohort). Together, these sources of error may have led to GWAS variant regression coefficients being under or over-estimated. These errors in turn may have contributed to the imprecision in the final causal effect estimate. Fifthly, information about gestational age was not available for UK Biobank participants, therefore self-reported BW in some individuals may have been small or large for their gestational age. However, an analysis by Horikoshi et al. [27] found no evidence for higher-than-expected heterogeneity in GWAS variant regression coefficients estimated in samples with vs. without the inclusion of gestational age as a covariate. This suggests that lack of information regarding gestational age did not introduce a systematic bias in the current study.

Conclusion

A Mendelian randomization analysis supported the hypothesis that BW within the normal range plays a causal role in refractive error development. A lower BW caused a modestly increased risk of myopia. The estimated causal effect of BW on refractive error was greater than that assessed from a conventional OLS analysis in the same sample, suggesting that confounding factors may buffer against the causal effect of BW on myopia.

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Table 1. Two-sample Mendelian randomization analysis for the role of birth weight in causing susceptibility to refractive error. The causal effect estimate is in units of Dioptres per 1 SD increase in BW. Summary statistics for stage 1 were from a meta-analysis of GWAS for BW in UK Biobank and EGG (n=188,039). Summary statistics for stage 2 were from a meta-analysis of GWAS for refractive error in UK Biobank and the CREAM (n=139,884).

Method	Estimate	95% CI		P-value
Simple median	0.15	-0.02	0.31	0.076
Weighted median	0.18	0.02	0.35	0.029
Penalized weighted median	0.20	0.03	0.36	0.020
IVW	0.15	0.00	0.30	0.044
Penalized IVW	0.21	0.08	0.33	0.002
Robust IVW	0.17	0.02	0.32	0.025
Penalized robust IVW	0.21	0.07	0.34	0.002
MR-Egger	0.18	-0.41	0.78	0.545
(intercept)	0.00	-0.02	0.01	0.918
Penalized MR-Egger	0.09	-0.42	0.61	0.720
(intercept)	0.00	-0.01	0.02	0.652
Robust MR-Egger	0.15	-0.36	0.66	0.564
(intercept)	0.00	-0.01	0.01	0.936
Penalized robust MR-Egger	0.08	-0.31	0.47	0.684
(intercept)	0.00	-0.01	0.01	0.557

Figure 1. Mendelian Randomization assumptions.

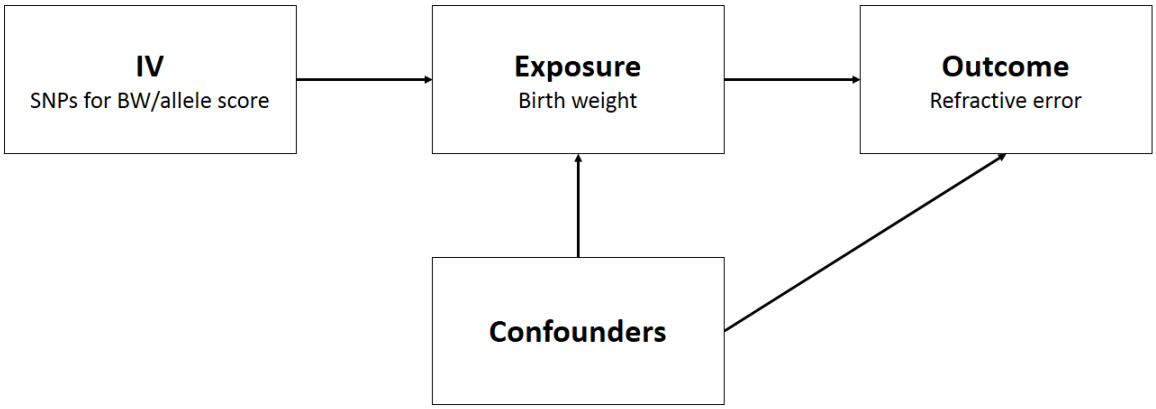

Figure 2. Selection of UK Biobank participants for GWAS for birth weight and for refractive error. Box outline colour corresponds to: black – GWAS for refractive error sample; red – OLS and MR sample; green – GWAS for birth weight sample. (Note that none of 95,504 participants in the refractive error GWAS sample had withdrawn consent to participate).

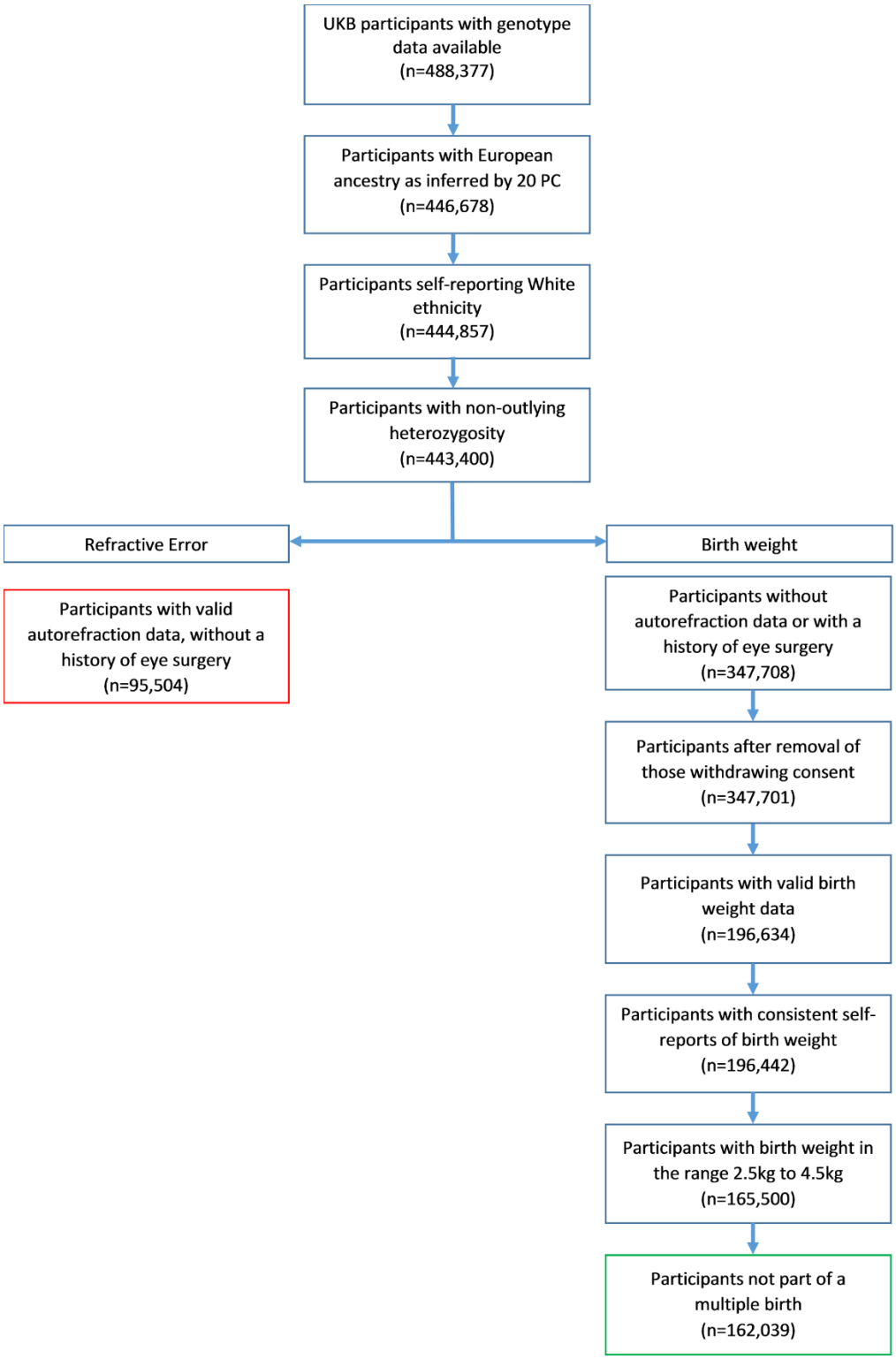

Figure 3. Comparison of estimated effect sizes for association with refractive error for 73 instrumental variables associated with birth weight. Error bars correspond to 95% confidence intervals. The solid green line and the dotted red line correspond to MR-Egger and IVW estimates respectively.

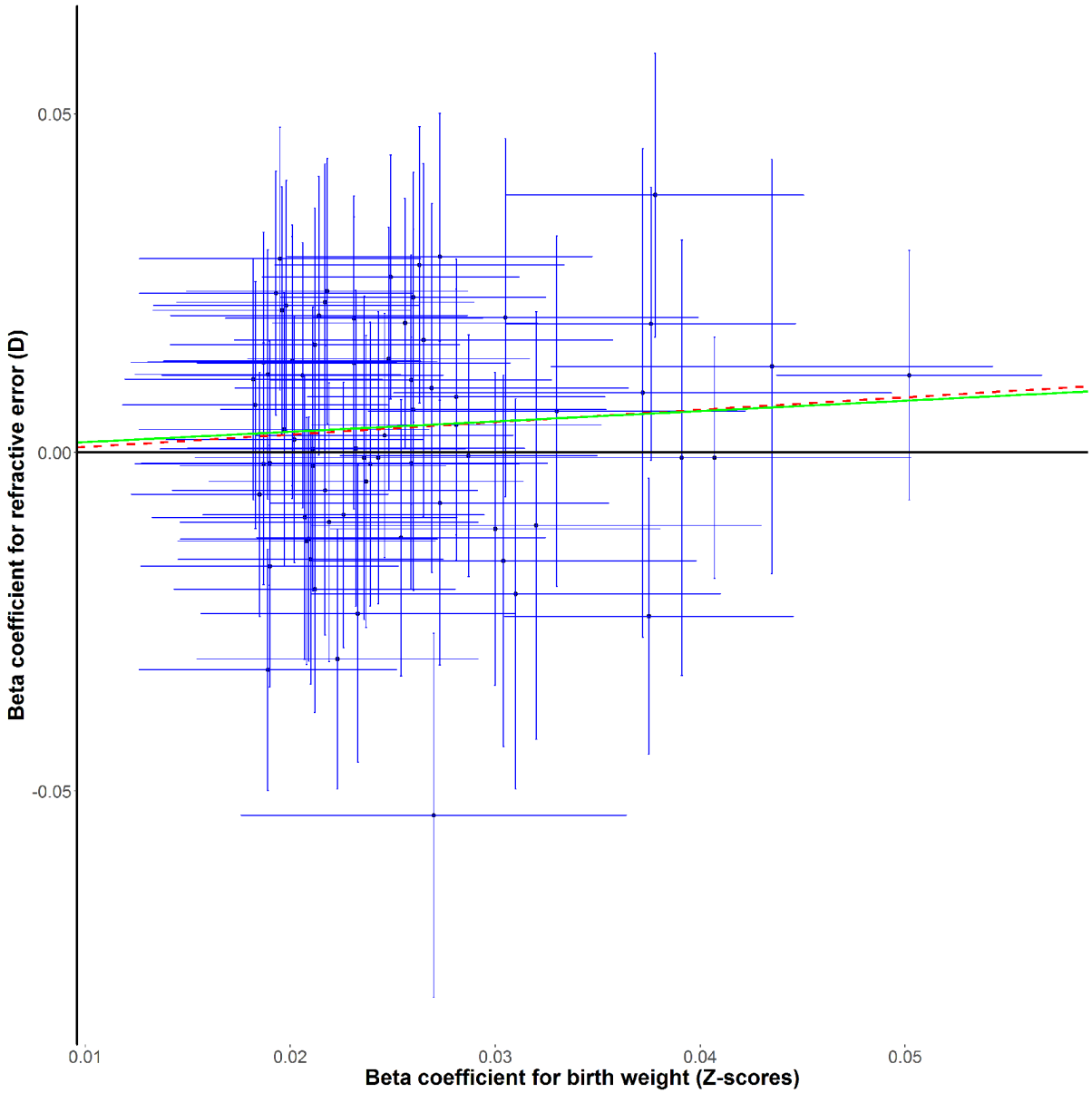

Figure 4. Interrelationships between birth weight and other traits. Solid arrows correspond to the direction of known association; dashed arrow corresponds to the tested association.