

Title: Mendelian Randomization evaluation of causal effects of fibrinogen on incident coronary heart disease

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1 **Abstract**

2 **Background:** Fibrinogen is an essential hemostatic factor and cardiovascular disease risk
3 factor. Early attempts at evaluating the causal effect of fibrinogen on coronary heart disease
4 (CHD) and myocardial infarction (MI) using Mendelian randomization (MR) used single
5 variant approaches, and did not take advantage of recent genome-wide association studies
6 (GWAS) or multi-variant, pleiotropy robust MR methodologies.

7 **Methods and Findings:** We evaluated evidence for a causal effect of fibrinogen on both
8 CHD and MI using MR. We used both an allele score approach and pleiotropy robust MR
9 models. The allele score was composed of 38 fibrinogen-associated variants from recent
10 GWAS. Initial analyses using the allele score incorporated data from 11 European-ancestry
11 prospective cohorts to examine incidence CHD and MI. We also applied 2 sample MR
12 methods with data from a prevalent CHD and MI GWAS. Results are given in terms of the
13 hazard ratio (HR) or odds ratio (OR), depending on the study design, and associated 95%
14 confidence interval (CI).

15 In single variant analyses no causal effect of fibrinogen on CHD or MI was observed. In
16 multi-variant analyses using incidence CHD cases and the allele score approach, the estimated
17 causal effect (HR) of a 1 g/L higher fibrinogen concentration was 1.62 (CI = 1.12, 2.36) when
18 using incident cases and the allele score approach. In 2 sample MR analyses that accounted
19 for pleiotropy, the causal estimate (OR) was reduced to 1.18 (CI = 0.98, 1.42) and 1.09 (CI =
20 0.89, 1.33) in the 2 most precise (smallest CI) models, out of 4 models evaluated. In the 2
21 sample MR analyses for MI, there was only very weak evidence of a causal effect in only 1
22 out of 4 models.

23 **Conclusions:** A small causal effect of fibrinogen on CHD is observed using multi-variant MR
24 approaches which account for pleiotropy, but not single variant MR approaches. Taken

1 together, results indicate that even with large sample sizes and multi-variant approaches MR
2 analyses still cannot exclude the null when estimating the causal effect of fibrinogen on CHD,
3 but that any potential causal effect is likely to be much smaller than observed in
4 epidemiological studies.

5

6 **Author Summary**

7 Initial Mendelian Randomization (MR) analyses of the causal effect of fibrinogen on coronary
8 heart disease (CHD) utilized single variants and did not take advantage of modern, multi-
9 variant approaches. This manuscript provides an important update to these initial analyses by
10 incorporating larger sample sizes and employing multiple, modern multi-variant MR
11 approaches to account for pleiotropy. We used incident cases to perform a MR study of the
12 causal effect of fibrinogen on incident CHD and the nested outcome of myocardial infarction
13 (MI) using an allele score approach. Then using data from a case-control genome-wide
14 association study for CHD and MI we performed two sample MR analyses with multiple,
15 pleiotropy robust approaches. Overall, the results indicated that associations between
16 fibrinogen and CHD in observational studies are likely upwardly biased from any underlying
17 causal effect. Single variant MR approaches show little evidence of a causal effect of
18 fibrinogen on CHD or MI. Multi-variant MR analyses of fibrinogen on CHD indicate there
19 may be a small positive effect, however this result needs to be interpreted carefully as the
20 95% confidence intervals were still consistent with a null effect. Multi-variant MR approaches
21 did not suggest evidence of even a small causal effect of fibrinogen on MI.

1 **Introduction:**

2 Fibrinogen is an essential component of the clotting and hemostasis system with a strong
3 genetic basis [1-3]. Although it primarily serves as the precursor to fibrin, it also carries out
4 several other functions, including enhancing platelet aggregation and mediating inflammation
5 [4, 5]. In epidemiologic studies, fibrinogen levels are associated with coronary heart disease
6 (CHD) [6-8], myocardial infarction (MI) [9, 10], ischemic stroke [11, 12], and abdominal
7 aortic aneurysm [13, 14].

8 Mendelian randomization (MR) is an instrumental variable analysis method which uses
9 genetic variants as instruments to uncover evidence for a causal relationship between a
10 modifiable risk factor and outcome.[15] MR studies utilizing a limited number of genetic
11 variants in the *FGB* promoter have yielded little evidence of a causal effect of fibrinogen on
12 CHD or MI [16-18]. In a genome-wide association study (GWAS) for fibrinogen, each
13 fibrinogen-associated variant was individually evaluated for association with CHD, but no
14 associations provided substantial evidence of a causal effect [19]. To date MR studies of
15 fibrinogen have been limited to single variant approaches which have not taken into account
16 recent GWAS findings or modern, multi-variant MR methodologies. Here we re-examine the
17 potential for fibrinogen to be a causal biomarker for CHD and MI, taking into account these
18 improved approaches.

19

20

21 **Results**

22 For incident CHD there were 3,147 incident events observed in 15,427 participants in the
23 discovery analyses, and 1,482 incident events among the 34,209 participants in the replication
24 analyses. Of the 18,798 participants in the incident MI discovery analyses, 1,711 had an
25 incident MI. For the replication analyses, there were 687 incident MI events out of the 33,288

1 participants. **Table 1** contains the distributions of clinical covariates and fibrinogen. The FGB
2 variant rs1800790 (commonly used in previous fibrinogen MR analyses) had a weaker
3 association (by effect size) than the allele score (**Supplemental Table 3**). In single variant
4 analyses of rs1800790 the estimated causal effect appeared to be centered around the null
5 with little evidence of a causal effect of fibrinogen on CHD or MI (**Supplemental Table 3**),
6 consistent with published literature. In multi-variant MR using the 2SC model, we observed
7 evidence of a causal association of fibrinogen on incident CHD in the discovery and
8 replication analyses which remained in a combined analysis of all cohorts (HR = 1.75; CI =
9 1.22–2.51; P = 0.002; **Figure 2**). For incident MI, we observed an elevated HR that included
10 the null, even in the combined analysis (HR = 1.45; CI = 0.85–2.49; P = 0.17; **Figure 3**).

11 *Pleiotropy robust models*

12 In sensitivity analyses four MR methods were used each of which is at least partially robust to
13 horizontal pleiotropy under differing assumptions. For CHD, three of the four models showed
14 a positive effect, albeit smaller than the effect observed in the 2SC model, with the MR
15 PRESSO method having the largest causal OR (OR = 1.18; CI = 0.98, 1.42; **Table 2**). For MI
16 only the MR PRESSO method showed a causal OR > 1 (OR = 1.16; CI = 0.98, 1.38; **Table**
17 **2**), again substantially reduced from that observed in the 2SC model. All other models for MI
18 showed little evidence of a causal effect of fibrinogen on MI.

19 As a further test we examined MR associations of fibrinogen on CHD risk using published
20 data available in the MR-Base. While some of the CHD risk factors showed a positive causal
21 effect estimate, none provided substantial evidence for excluding the null after accounting for
22 the number of tests performed (**Supplemental Table 5**).

23

24 **Discussion**

1 The attractiveness of fibrinogen as a causal factor in CHD comes from its roles in both
2 thrombosis and inflammation. Fibrinogen is the precursor to fibrin, which interlinks into a
3 mesh that acts as the scaffold of blood clots. Additionally, fibrinogen also has an active role
4 in platelet aggregation,[20] thus contributing to the formation of platelet plugs. By binding the
5 CD11b/CD18 integrin receptor fibrinogen activates the NF- κ B pathway [5], an important
6 pathway in inflammation as well as the formation, destabilization, and rupture of
7 atherosclerotic plaques [21, 22]. As a modifiable risk factor [23] even a small causal effect of
8 fibrinogen on CHD could have substantial public health implications.

9 Using the allele score approach, a 1 g/L higher fibrinogen concentration was causally
10 associated with a HR of 1.75 (CI = 1.22–2.51) in the combined cohort analysis for CHD.
11 However, sensitivity analysis using methods robust to pleiotropy arising from independent
12 effects of SNPs on exposure and outcome (which could invalidate MR analyses) suggested a
13 substantially weaker causal effect on CHD even for the model with the strongest effect
14 estimate (OR = 1.18 per 1 g/L higher fibrinogen; CI = 0.98, 1.42), and the MR Egger model
15 showed virtually no evidence of a causal effect – though the wide 95% confidence interval
16 encompassed effects from all other models. Overall, when accounting for potential horizontal
17 pleiotropy, the accumulated evidence points to a substantially weaker casual effect of
18 fibrinogen on CHD than the observational risk ratio of 1.8 (CI = 1.6, 2.0) previously reported
19 [6]. Using rs1800790 in a single variant MR analysis, there was limited evidence of any
20 causal effect, though the 95% confidence interval could not exclude positive estimated causal
21 effects seen in multi-variant analyses. In combination these analyses suggest that when after
22 accounting for horizontal pleiotropy the effect of fibrinogen on CHD is likely to be small and
23 that current MR estimates of the potential causal effect remain unable to exclude the null
24 despite large sample sizes and the latest methodologies.

25 ***Comparison with previous MR analyses***

1 Previous MR studies assessing the causal effect of fibrinogen on CHD or MI focused
2 exclusively on rs1800790 [24, 25]. In a few studies one additional variant also in the *FGB*
3 promoter region was examined, however this variant is in nearly complete LD with
4 rs1800790, particularly in Europeans [16, 18]. The allele score was a better predictor of
5 fibrinogen than rs1800790 alone (**Supplemental Table 3**). Though the allele score estimated
6 a causal effect of fibrinogen on CHD similar to observational studies, much of this appeared
7 to be driven by pleiotropy as estimated effects decreased in models more robust to pleiotropy
8 (**Table 2**). This highlights the need to balance increased power from multi-variant approaches
9 with the potential for increased pleiotropy in these instruments.

10 For the CHARGE cohorts we used exclusively incident cases whereas previous studies
11 utilized populations composed entirely or primarily of prevalent cases. In some instances, the
12 use of prevalent cases may bias MR studies such as if the disease subsequently what is
13 perceived as a disease risk factor, e.g. if CHD leads to higher fibrinogen as opposed to the
14 reverse, then reverse confounding can still occur even in an MR setting [26]. Additionally, if
15 the risk factor were to affect severity of an event, e.g. the fatality of MI, then use of prevalent
16 cases may dilute the MR-estimated causal effect as the most severe cases may not be observed
17 due to being too ill to participate or suffering a fatal event. This type of prevalence-incidence
18 bias is not exclusive to MR analyses [27-29]. However, care must still be taken when
19 interpreting results from incident case MR studies as the exclusion of prevalent cases is
20 equivalent to conditioning on disease status at baseline. This has the potential to introduce
21 bias in the form of an exclusion restriction violation.[30] Whether bias is introduced and the
22 degree of confounding are dependent on the actual biological processes that account for the
23 relationship between the genetic instrument(s) chosen, the modifiable risk factor, and outcome
24 in the MR analysis. When performing incident case MR it is best to combine the efforts with

1 MR analyses including prevalent cases and interpret results for both with careful
2 consideration towards their underlying assumptions, strengths, and weaknesses.
3 In general, our results are compatible with previous MR studies, however we use more
4 modern methods, including multi-variant, pleiotropy robust methods, able to produce smaller
5 confidence intervals and which indicate that after accounting for pleiotropy there may be a
6 small positive effect of fibrinogen on CHD. This is particularly true for the methods
7 producing the most precise estimates. However, these results warrant further investigations as
8 confidence intervals for some models were still wide and with results for the single variant
9 and MR Egger analyses possibly more consistent with no causal effect than even a small
10 causal effect.

11 ***Strengths and limitations***

12 As with all MR studies the causal effects estimated here are based on regression estimates for
13 genetic variants and are only valid, causal estimates under the assumptions of MR.
14 Additionally, causal estimates generated via MR methodologies are for lifelong, genetically
15 determined increases in the exposure, e.g. fibrinogen, which means that caution should be
16 exercised when applying clinical interpretations or attempting to translate results into
17 estimates of an intervention.[31, 32]This study had some overlap between studies involved in
18 the GWAS used to select fibrinogen variants and those used in the MR analyses. Our
19 approach to mitigate this was to replicate the allele score analysis in an independent set of
20 cohorts. For the pleiotropy robust 2-sample MR approaches this overlap was unavoidable,
21 however there was no overlap for the cases which means that unbiased estimates should be
22 obtained [33]. A strength of the study is the use of incident cases for the allele score model
23 approach which reduces the potential for bias from reverse confounding (which can still affect
24 MR studies) and prevalence-incidence bias. Additionally, even though the allele score
25 approach was sensitive to horizontal pleiotropy we used an array of additional approaches that

1 were each partially robust to horizontal pleiotropy through different assumptions about the
2 nature of the pleiotropy. These models often have lower power than other approaches, which
3 motivated our use of a previously published GWAS which had 60,801 prevalent cases and
4 123,504 controls [34]. However, to prevent potential bias and more closely align with our
5 initial analyses, a large sample size of incident cases independent of those used to evaluate
6 associations between genetic variants and fibrinogen would have been preferable.

7 **Conclusion**

8 Fibrinogen represents an important role in thrombosis, platelet aggregation, and inflammation
9 making it a promising risk factor for CHD. Despite the epidemiological evidence, MR studies
10 using prevalent cases and single variant approaches have consistently shown no causal effect
11 of fibrinogen on CHD. Our results indicate that epidemiologic studies may substantially over-
12 estimate any causal effect of fibrinogen on CHD. While some MR models which accounted
13 for pleiotropy did show a modest causal effect, the 95% confidence intervals still contained
14 the null indicating that researchers should exercise caution in interpreting these results.
15 Further analyses using larger sample sizes and more precise methods are warranted to better
16 resolve the effect of fibrinogen on CHD.

17

18 **Methods:**

19 This study was conducted within the Cohorts for Heart and Aging Research in Genomic
20 Epidemiology (CHARGE) consortium [35] using 11 European-ancestry cohorts. For incident
21 CHD, six cohorts participated in the initial (discovery) analyses (N = 15,427), and four
22 cohorts (N = 34,209) contributed data for replication. For incident MI, six cohorts participated
23 in the discovery (N = 18,798), and three cohorts participated in the replication (N = 33,288)
24 analyses. Details on all cohorts are given in the Supplemental Materials and the clinical
25 covariates in **Table 1**, and **Figure 1** outlines all analyses. Data collection analysis for all

1 cohorts was approved by their respective Institutional Review Boards and/or ethical
2 committees, and all cohorts obtained written, informed consent from participants.

3 ***Assessment of CHD and MI***

4 We defined incident CHD as validated, incident fatal or non-fatal CHD events which
5 included: validated hospitalized MI, CHD-related hospitalizations, definite CHD deaths,
6 likely CHD deaths, and CHD-related revascularization procedures, e.g. percutaneous coronary
7 intervention and coronary artery bypass grafting. Incident MI was defined as a validated fatal
8 or non-fatal MI and included definite MI hospitalizations. For cohorts that used questionnaires
9 as a component of the follow-up procedures, all events were corroborated with medical
10 records and/or review by trained medical personnel. Cohort specific details are given in the
11 Supplemental Online Methods.

12 ***Fibrinogen Assessment***

13 Fibrinogen was assessed by a variety of methods, with seven cohorts using the Clauss method
14 [36]. Of the remaining four cohorts, RS used a clotting time-derived method to assess
15 fibrinogen concentrations, while KORA, MESA, and WGHS used immunological assays to
16 assess total fibrinogen.

17 ***Genotyping and Imputation***

18 Genotyping and imputation were performed separately in all cohorts, per published methods
19 [37]. All participating studies used either the HapMap build 36 [38], 1000 Genomes phase I
20 version 3, or 1000 Genomes phase I version 2 reference panel for imputation [39]. Imputation
21 was performed via MACH[40] or IMPUTE [41]. Low quality variants were excluded in line
22 with previously published approaches: MACH imputation quality < 0.3 or IMPUTE
23 imputation quality < 0.4 [37].

24 ***Creation of the Allele Score***

1 We evaluated 69 variants associated with fibrinogen in at least one of three recent
2 genome/exome-wide association studies [2, 19, 37] for inclusion into the allele score [42]. We
3 applied four criteria to each variant to improve the plausibility that each meets the MR
4 assumptions. First, to ensure that the variants were not correlated with known risk factors for
5 cardiovascular disease (CVD), the Spearman correlation between each of the variants and
6 body mass index (BMI), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein
7 (HDL) cholesterol, type 2 diabetes mellitus (binary), hypertension (binary), and smoking
8 (ever, never, current) was tested within each cohort and any variants with a Spearman
9 correlation greater than 0.10 in any cohort for any of these outcomes were removed. Second,
10 the variants were tested for linkage disequilibrium (LD) with known CHD loci [34, 43-52]
11 using SNAP from the Broad Institute with LD patterns coming from European ancestry
12 individuals [53]. As no variant had $r^2 > 0.20$ with a CHD locus, they were considered
13 independent of known CHD loci. Next, we reduced pairs of variants in high LD ($r^2 > 0.70$) by
14 preferentially retaining those variants that were found in the largest genome-wide scan [37].
15 Finally, we eliminated any variants that were missing across any of the discovery cohorts,
16 leaving 38 variants that composed the allele score (**Supplemental Table 1**). We tested the
17 allele score for association with each of the aforementioned CHD risk factors in each cohort
18 as well as in a meta-analysis of all cohorts. The allele score was not associated with any CHD
19 risk factor in the meta-analysis after a Bonferroni correction for the six tests performed ($P >$
20 0.008 ; **Supplemental Table 2**). Six variants from the allele score which were unavailable in
21 one or more replication cohorts were removed from the allele score in the replication phase to
22 ensure a consistent allele score in the replication meta-analysis (**Supplemental Table 1**). In a
23 sensitivity analysis these variants were also removed from the discovery cohorts and the
24 causal effect evaluated in a combined meta-analysis.

1 Each genotype was aligned prior to summing to create the score so that the designated effect
2 allele corresponded to a positive association with fibrinogen according to the direction of
3 effect in the largest and most recent fibrinogen GWAS [19].

4 ***Mendelian Randomization***

5 MR is a powerful framework that uses genetic variants as instrumental variables to infer
6 causal relationships between a defined exposure and outcome. The causal effect estimated by
7 MR is the alteration in exposure due to genetic variation and is thus assumed to be over the
8 entire life course. There are three assumptions for a genetic variant to be a valid instrument
9 for MR[54]

- 10 1. The genetic variant is independent of confounders of exposure and outcome under
11 examination
- 12 2. The genetic variant is associated with the exposure
- 13 3. The genetic variant is independent of the outcome conditional on the exposure and any
14 confounders

15 In addition to these three conditions, valid estimates from MR are dependent on any
16 parametric assumptions of the model being used to estimate relevant coefficients and standard
17 errors.

18 Our initial MR analyses used a two-stage procedure employing a Cox regression model
19 (2SC). To improve power, we regressed fibrinogen on age and sex and used the resulting
20 residuals as input to the 2SC analyses. In the first stage of the 2SC procedure the fibrinogen
21 residuals were regressed on the allele score. In the second stage the predicted values from the
22 first stage regression were associated with incident MI or CHD via a Cox proportional
23 hazards model. This approach is similar to the two-stage predictor-substitution MR approach
24 [55-57], and results from the 2SC model are given per unit (g/L) increase in the fibrinogen
25 residuals. We used a fixed effects model for all meta-analyses since we observed little

1 heterogeneity according to the Q-statistics [58] ($P(Q) > 0.05$ for all analyses). We also
2 compared associations with our allele score to those obtained using a single variant, FGB -
3 455G>A (rs1800790), which is a commonly used variant for fibrinogen MR analyses [16, 18].
4 We performed sensitivity analyses using four pleiotropy robust methods each of which uses a
5 different approach to partially relax the no horizontal pleiotropy assumption of MR
6 analyses:[59] MR-Egger [54], MR mode based estimate (MBE),[60] MR PRESSO,[61] and
7 Weighted median [62]. For these sensitivity analyses, we used the prevalent CHD and MI
8 GWAS results from CARDIoGRAMplusC4D consortium [34] as it had a larger sample size
9 (60,801 prevalent cases and 123,504 controls) and these methods often have lower power to
10 detect effects. For estimates of variant effects on fibrinogen we used fixed-effects meta-
11 analysis estimates from the 11 cohorts in these analyses. Since an individual cannot be both a
12 prevalent and incident CHD or MI case at the same sampling, there was no overlap amongst
13 the cases between our incident analyses and the prevalent cases used in the
14 CARDIoGRAMplusC4D GWAS. There would still be some overlap amongst the non-
15 cases/controls which could bias estimates towards the null.

16 We also examined whether fibrinogen showed evidence for a causal effect on 7 metabolic
17 CHD risk factors using MR-base (www.mrbase.org), a database of published GWAS
18 available for MR [63]. We focused on metabolic CHD risk factors as initial results indicated
19 that body mass index was the trait with which our allele score showed the strongest evidence
20 for pleiotropy - potentially horizontal (i.e. SNPs affecting fibrinogen and CHD via
21 independent pathways) and vertical (i.e. fibrinogen-associated SNPs also associated with risk
22 factors downstream of fibrinogen) as the associations did not distinguish between the two.

23 The CHD risk factors were body mass index [64], waist circumference [65], waist-to-hip ratio
24 [65], low-density lipoprotein cholesterol,[66] triglycerides [66], homeostatic model
25 assessment insulin resistance (HOMA-IR) [67], and Type 2 diabetes [68]. As MR-base only

1 contains published GWAS we used the most recently published GWAS for fibrinogen for our
2 variant-fibrinogen associations [1] but limited to those variants present in our allele score. For
3 the CHD risk factors we compared causal effect estimates obtained from the inverse variance
4 weighted method (which assumes no unbalanced horizontal pleiotropy), to those from the
5 pleiotropy robust MR Egger, and Weighted median methods. All three methodologies were
6 implemented in MR-base.

7 Statistical analyses were performed in R.[69] Meta-analyses were performed using the R
8 package *metafor*. [70] Cox models were estimated via the *coxph* function in the R package
9 survival[71] with the exception of SHIP where the *survreg* function was used with an
10 exponential distribution to account for the interval censored data. MR-Egger and weighted
11 median results were performed using the R package *MendelianRandomization* and *Two*
12 *Sample MR*. [63] MR MBE analyses were performed using the methods given by Hartwig *et*
13 *al.* [60] The default bandwidth ($\phi = 1$) was used for MR MBE as results did not show
14 sensitivity to the choice of bandwidth. MR PRESSO analyses were performed using code
15 available at the MR PRESSO GitHub repository (<https://github.com/rondolab/MR-PRESSO>)
16 [61]. We used the robust MR estimates from MR PRESSO which are equivalent to
17 performing an inverse-variance weighted MR analysis after removing outlying variants,
18 which may be influenced by horizontal pleiotropy, as identified by MR PRESSO. Results
19 from the 2SC model are reported in terms of the hazard ratio (HR), while all results that
20 utilize the prevalent disease GWAS are reported in terms of the odds ratio (OR). All HR and
21 OR are given per 1 g/L higher fibrinogen. All confidence intervals (CI) reported are 95% CI.

22

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4

5 **Author Contributions**

6 JIR, CJO'D, MD, DMB, LCB, JWK, CKW-C, PMR, DIC, SLRK, NLS, A Peters, ACM,
7 ADJ, and WM contributed to the study concept and design. KDT, Y-DIC, RT, JIR, OHF,
8 MD, A Petersman, Y-PF, DMB, RAM, LCB, WK, JWK, PMR, DIC, TM, PAP, JAS, SLRK,
9 MEK, GD, MW, KS, MM-N, EB, MPMdeM, NS, AGU, WM, and BMP performed data
10 acquisition. Data analysis was performed by XG, JY, TK, SG, Y-PF, LRY, CKW-C, FG,
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18

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1 **References**

- 2 1. de Vries PS, Chasman DI, Sabater-Lleal M, Chen MH, Huffman JE, Steri M, et al. A
3 meta-analysis of 120 246 individuals identifies 18 new loci for fibrinogen concentration. *Hum*
4 *Mol Genet.* 2016;25(2):358-70. Epub 2015/11/13. doi: 10.1093/hmg/ddv454. PubMed PMID:
5 26561523; PubMed Central PMCID: PMC4715256.
- 6 2. Huffman JE, de Vries PS, Morrison AC, Sabater-Lleal M, Kacprowski T, Auer PL, et
7 al. Rare and low-frequency variants and their association with plasma levels of fibrinogen,
8 FVII, FVIII, and vWF. *Blood.* 2015. doi: 10.1182/blood-2015-02-624551.
- 9 3. Sabater-Lleal M, Huang J, Chasman D, Naitza S, Dehghan A, Johnson AD, et al.
10 Multiethnic meta-analysis of genome-wide association studies in >100 000 subjects identifies
11 23 fibrinogen-associated Loci but no strong evidence of a causal association between
12 circulating fibrinogen and cardiovascular disease. *Circulation.* 2013;128(12):1310-24. Epub
13 2013/08/24. doi: 10.1161/circulationaha.113.002251. PubMed PMID: 23969696; PubMed
14 Central PMCID: PMC3842025.
- 15 4. Mikhailidis DP, Barradas MA, Maris A, Jeremy JY, Dandona P. Fibrinogen mediated
16 activation of platelet aggregation and thromboxane A2 release: pathological implications in
17 vascular disease. *Journal of clinical pathology.* 1985;38(10):1166-71. Epub 1985/10/01.
18 PubMed PMID: 3902901; PubMed Central PMCID: PMC499462.
- 19 5. Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease.
20 *Seminars in Immunopathology.* 2012;34(1):43-62. doi: 10.1007/s00281-011-0290-8.
- 21 6. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, c-reactive protein,
22 albumin, or leukocyte count with coronary heart disease: Meta-analyses of prospective
23 studies. *JAMA.* 1998;279(18):1477-82. doi: 10.1001/jama.279.18.1477.

- 1 7. de Maat MP, Pietersma A, Kofflard M, Sluiter W, Kluft C. Association of plasma
2 fibrinogen levels with coronary artery disease, smoking and inflammatory markers.
3 *Atherosclerosis*. 1996;121(2):185-91.
- 4 8. Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, et al. β Fibrinogen Gene
5 Polymorphisms Are Associated With Plasma Fibrinogen and Coronary Artery Disease in
6 Patients With Myocardial Infarction. The ECTIM Study. 1996;93(3):440-9. doi:
7 10.1161/01.cir.93.3.440.
- 8 9. Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring Plasma Fibrinogen to
9 Predict Stroke and Myocardial Infarction: An Update. *Arteriosclerosis, Thrombosis, and*
10 *Vascular Biology*. 1999;19(6):1368-77. doi: 10.1161/01.atv.19.6.1368.
- 11 10. Ma J, Hennekens CH, Ridker PM, Stampfer MJ. A prospective study of fibrinogen
12 and risk of myocardial infarction in the physicians' health study. *Journal of the American*
13 *College of Cardiology*. 1999;33(5):1347-52. doi: 10.1016/S0735-1097(99)00007-8.
- 14 11. Rothwell PM, Howard SC, Power DA, Gutnikov SA, Algra A, van Gijn J, et al.
15 Fibrinogen Concentration and Risk of Ischemic Stroke and Acute Coronary Events in 5113
16 Patients With Transient Ischemic Attack and Minor Ischemic Stroke. *Stroke*.
17 2004;35(10):2300-5. doi: 10.1161/01.STR.0000141701.36371.d1.
- 18 12. Chuang S-Y, Bai C-H, Chen W-H, Lien L-M, Pan W-H. Fibrinogen Independently
19 Predicts the Development of Ischemic Stroke in a Taiwanese Population: CVDFACTS Study.
20 *Stroke*. 2009;40(5):1578-84. doi: 10.1161/strokeaha.108.540492.
- 21 13. Singh K, Bønaa KH, Jacobsen BK, Bjørk L, Solberg S. Prevalence of and Risk Factors
22 for Abdominal Aortic Aneurysms in a Population-based Study: The Tromsø Study. *American*
23 *Journal of Epidemiology*. 2001;154(3):236-44. doi: 10.1093/aje/154.3.236.

- 1 14. Al-Barjas HS, Ariëns R, Grant P, Scott JA. Raised Plasma Fibrinogen Concentration
2 in Patients With Abdominal Aortic Aneurysm. *Angiology*. 2006;57(5):607-14. doi:
3 10.1177/0003319706293132.
- 4 15. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology
5 contribute to understanding environmental determinants of disease?*. *International Journal of*
6 *Epidemiology*. 2003;32(1):1-22. doi: 10.1093/ije/dyg070.
- 7 16. Keavney B, Danesh J, Parish S, Palmer A, Clark S, Youngman L, et al. Fibrinogen and
8 coronary heart disease: test of causality by 'Mendelian randomization'. *International Journal*
9 *of Epidemiology*. 2006;35(4):935-43.
- 10 17. Davey Smith G, Harbord R, Ebrahim S. Fibrinogen, C-reactive protein and coronary
11 heart disease: does Mendelian randomization suggest the associations are non-causal? *Qjm*.
12 2004;97(3):163-6.
- 13 18. Davey Smith G, Harbord R, Milton J, Ebrahim S, Sterne JA. Does elevated plasma
14 fibrinogen increase the risk of coronary heart disease? Evidence from a meta-analysis of
15 genetic association studies. *Arteriosclerosis, thrombosis, and vascular biology*.
16 2005;25(10):2228-33.
- 17 19. Sabater-Lleal M, Huang J, Chasman DI, Naitza S, Dehghan A, Johnson AD, et al. A
18 Multi-Ethnic Meta-Analysis of Genome-Wide Association Studies in over 100,000 subjects
19 identifies 23 fibrinogen-associated loci but no strong evidence of a causal association between
20 circulating fibrinogen and cardiovascular disease. *Circulation*. 2013:CIRCULATIONAHA.
21 113.002251.
- 22 20. Bennett JS. Platelet-fibrinogen interactions. *Annals of the New York Academy of*
23 *Sciences*. 2001;936:340-54. Epub 2001/07/20. PubMed PMID: 11460491.

- 1 21. Ross R. Atherosclerosis--an inflammatory disease. *The New England journal of*
2 *medicine*. 1999;340(2):115-26. Epub 1999/01/14. doi: 10.1056/nejm199901143400207.
3 PubMed PMID: 9887164.
- 4 22. Pamukcu B, Lip GY, Shantsila E. The nuclear factor--kappa B pathway in
5 atherosclerosis: a potential therapeutic target for atherothrombotic vascular disease.
6 *Thrombosis research*. 2011;128(2):117-23. Epub 2011/06/04. doi:
7 10.1016/j.thromres.2011.03.025. PubMed PMID: 21636112.
- 8 23. Kamath S, Lip GYH. Fibrinogen: biochemistry, epidemiology and determinants. *QJM*.
9 2003;96(10):711-29. doi: 10.1093/qjmed/hcg129.
- 10 24. Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, Abildgaard S, Schnohr P,
11 Nordestgaard BG. A common mutation (G-455--> A) in the beta-fibrinogen promoter is an
12 independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of
13 9,127 individuals based on the Copenhagen City Heart Study. *Journal of Clinical*
14 *Investigation*. 1997;99(12):3034-9. PubMed PMID: PMC508156.
- 15 25. Leander K, Wiman B, Hallqvist J, Falk G, De Faire U. The G-455A polymorphism of
16 the fibrinogen BB-gene relates to plasma fibrinogen in male cases, but does not interact with
17 environmental factors in causing myocardial infarction in either men or women. *Journal of*
18 *internal medicine*. 2002;252(4):332-41.
- 19 26. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal
20 inference in epidemiological studies. *Human Molecular Genetics*. 2014;23(R1):R89-R98. doi:
21 10.1093/hmg/ddu328.
- 22 27. Neyman J. Statistics—Servant of All Science. *Science*. 1955;122(3166):401-6. doi:
23 10.1126/science.122.3166.401.
- 24 28. Hill G, Connelly J, Hebert R, Lindsay J, Millar W. Neyman's bias re-visited. *J Clin*
25 *Epidemiol*. 2003;56(4):293-6. Epub 2003/05/28. PubMed PMID: 12767404.

- 1 29. Delgado-Rodríguez M, Llorca J. Bias. *Journal of Epidemiology and Community*
2 *Health*. 2004;58(8):635-41. doi: 10.1136/jech.2003.008466.
- 3 30. Paternoster L, Tilling K, Davey Smith G. Genetic epidemiology and Mendelian
4 randomization for informing disease therapeutics: Conceptual and methodological challenges.
5 *PLOS Genetics*. 2017;13(10):e1006944. doi: 10.1371/journal.pgen.1006944.
- 6 31. O'Donnell CJ. Mendelian randomization evidence for cardiovascular precision
7 medicine. *JAMA Cardiology*. 2018;3(7):627-8. doi: 10.1001/jamacardio.2018.1543.
- 8 32. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic
9 disease: challenges in evaluating causality. *Nature Reviews Cardiology*. 2017;14:577. doi:
10 10.1038/nrcardio.2017.78.
- 11 33. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample
12 Mendelian randomization. *Genetic Epidemiology*. 2016;40(7):597-608. doi:
13 doi:10.1002/gepi.21998.
- 14 34. The CARDIoGRAMplusC4D Consortium. A comprehensive 1000 Genomes-based
15 genome-wide association meta-analysis of coronary artery disease. *Nat Genet*.
16 2015;47(10):1121-30. doi: 10.1038/ng.3396
17 <http://www.nature.com/ng/journal/v47/n10/abs/ng.3396.html#supplementary-information>.
- 18 35. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al.
19 Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium:
20 Design of prospective meta-analyses of genome-wide association studies from 5 cohorts.
21 *Circulation Cardiovascular genetics*. 2009;2(1):73-80. Epub 2009/12/25. doi:
22 10.1161/circgenetics.108.829747. PubMed PMID: 20031568; PubMed Central PMCID:
23 PMCPmc2875693.

- 1 36. Clauss A. Gerinnungsphysiologische schnellmethode zur bestimmung des fibrinogens.
2 Acta haematologica. 1957;17(4):237-46.
- 3 37. De Vries PS, Chasman DI, Sabater-Lleal M, Chen M-H, Huffman JE, Steri M, et al. A
4 meta-analysis of 120,246 individuals identifies 18 new loci for fibrinogen concentration.
5 Human molecular genetics. 2015:ddv454.
- 6 38. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second
7 generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449(7164):851-61.
- 8 39. Genomes Project Consortium. An integrated map of genetic variation from 1,092
9 human genomes. Nature. 2012;491(7422):56-65.
- 10 40. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: Using Sequence and
11 Genotype Data to Estimate Haplotypes and Unobserved Genotypes. Genetic epidemiology.
12 2010;34(8):816-34. doi: 10.1002/gepi.20533. PubMed PMID: PMC3175618.
- 13 41. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation
14 method for the next generation of genome-wide association studies. PLoS Genet.
15 2009;5(6):e1000529.
- 16 42. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian
17 randomization. International Journal of Epidemiology. 2013;42(4):1134-44. doi:
18 10.1093/ije/dyt093.
- 19 43. Lettre G, Palmer CD, Young T, Ejebe KG, Allayee H, Benjamin EJ, et al. Genome-
20 wide association study of coronary heart disease and its risk factors in 8,090 African
21 Americans: the NHLBI CARE Project. PLoS Genet. 2011;7(2):e1001300.
- 22 44. Carty CL, Buzkova P, Fornage M, Franceschini N, Cole S, Heiss G, et al. Associations
23 between incident ischemic stroke events and stroke and cardiovascular disease-related
24 genome-wide association studies single nucleotide polymorphisms in the Population
25 Architecture Using Genomics and Epidemiology study. Circulation Cardiovascular genetics.

- 1 2012;5(2):210-6. Epub 2012/03/10. doi: 10.1161/circgenetics.111.962191. PubMed PMID:
2 22403240; PubMed Central PMCID: PMCPmc3402178.
- 3 45. Chan K, Patel RS, Newcombe P, Nelson CP, Qasim A, Epstein SE, et al. Association
4 between the chromosome 9p21 locus and angiographic coronary artery disease burden: a
5 collaborative meta-analysis. *J Am Coll Cardiol.* 2013;61(9):957-70. Epub 2013/01/29. doi:
6 10.1016/j.jacc.2012.10.051. PubMed PMID: 23352782; PubMed Central PMCID:
7 PMCPmc3653306.
- 8 46. Cheng YC, Anderson CD, Bione S, Keene K, Maguire JM, Nalls M, et al. Are
9 myocardial infarction--associated single-nucleotide polymorphisms associated with ischemic
10 stroke? *Stroke.* 2012;43(4):980-6. Epub 2012/03/01. doi: 10.1161/strokeaha.111.632075.
11 PubMed PMID: 22363065; PubMed Central PMCID: PMCPmc3622211.
- 12 47. Dichgans M, Malik R, Konig IR, Rosand J, Clarke R, Gretarsdottir S, et al. Shared
13 genetic susceptibility to ischemic stroke and coronary artery disease: a genome-wide analysis
14 of common variants. *Stroke.* 2014;45(1):24-36. Epub 2013/11/23. doi:
15 10.1161/strokeaha.113.002707. PubMed PMID: 24262325; PubMed Central PMCID:
16 PMCPmc4112102.
- 17 48. Lieb W, Jansen H, Loley C, Pencina MJ, Nelson CP, Newton-Cheh C, et al. Genetic
18 predisposition to higher blood pressure increases coronary artery disease risk. *Hypertension*
19 *(Dallas, Tex : 1979).* 2013;61(5):995-1001. Epub 2013/03/13. doi:
20 10.1161/hypertensionaha.111.00275. PubMed PMID: 23478099; PubMed Central PMCID:
21 PMCPmc3855241.
- 22 49. Patel RS, Ye S. Genetic determinants of coronary heart disease: new discoveries and
23 insights from genome-wide association studies. *Heart (British Cardiac Society).*
24 2011;97(18):1463-73. Epub 2011/07/28. doi: 10.1136/hrt.2010.219675. PubMed PMID:
25 21791514.

- 1 50. Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary
2 artery disease: focus on genetic risk factors and identification of genetic variants. *The*
3 *Application of Clinical Genetics*. 2014;7:15-32. doi: 10.2147/TACG.S35301. PubMed PMID:
4 PMC3920464.
- 5 51. Zhang X, Johnson AD, Hendricks AE, Hwang SJ, Tanriverdi K, Ganesh SK, et al.
6 Genetic associations with expression for genes implicated in GWAS studies for
7 atherosclerotic cardiovascular disease and blood phenotypes. *Hum Mol Genet*.
8 2014;23(3):782-95. Epub 2013/09/24. doi: 10.1093/hmg/ddt461. PubMed PMID: 24057673;
9 PubMed Central PMCID: PMCPmc3900869.
- 10 52. Roberts R, Stewart AF. Genes and coronary artery disease: where are we? *J Am Coll*
11 *Cardiol*. 2012;60(18):1715-21. Epub 2012/10/09. doi: 10.1016/j.jacc.2011.12.062. PubMed
12 PMID: 23040572.
- 13 53. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI.
14 SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap.
15 *Bioinformatics (Oxford, England)*. 2008;24(24):2938-9. Epub 2008/11/01. doi:
16 10.1093/bioinformatics/btn564. PubMed PMID: 18974171; PubMed Central PMCID:
17 PMCPmc2720775.
- 18 54. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid
19 instruments: effect estimation and bias detection through Egger regression. *International*
20 *Journal of Epidemiology*. 2015;44(2):512-25. doi: 10.1093/ije/dyv080.
- 21 55. Burgess S. Identifying the odds ratio estimated by a two-stage instrumental variable
22 analysis with a logistic regression model. *Statistics in medicine*. 2013;32(27):4726-47. Epub
23 2013/06/05. doi: 10.1002/sim.5871. PubMed PMID: 23733419; PubMed Central PMCID:
24 PMCPmc3935453.

- 1 56. Didelez V, Meng S, Sheehan NA. Assumptions of IV methods for observational
2 epidemiology. *Statistical Science*. 2010;22-40.
- 3 57. Dixon SC, Nagle CM, Thrift AP, Pharoah PD, Pearce CL, Zheng W, et al. Adult body
4 mass index and risk of ovarian cancer by subtype: a Mendelian randomization study.
5 *International Journal of Epidemiology*. 2016;45(3):884-95. doi: 10.1093/ije/dyw158.
- 6 58. Cochran WG. The Comparison of Percentages in Matched Samples. *Biometrika*.
7 1950;37(3/4):256-66. doi: 10.2307/2332378.
- 8 59. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in
9 Mendelian randomization studies. *Human Molecular Genetics*. 2018;27(R2):R195-R208. doi:
10 10.1093/hmg/ddy163.
- 11 60. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian
12 randomization via the zero modal pleiotropy assumption. *International Journal of*
13 *Epidemiology*. 2017;46(6):1985-98. doi: 10.1093/ije/dyx102.
- 14 61. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal
15 pleiotropy in causal relationships inferred from Mendelian randomization between complex
16 traits and diseases. *Nature Genetics*. 2018;50(5):693-8. doi: 10.1038/s41588-018-0099-7.
- 17 62. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in
18 Mendelian Randomization with Some Invalid Instruments Using a Weighted Median
19 Estimator. *Genetic Epidemiology*. 2016;40(4):304-14. doi: doi:10.1002/gepi.21965.
- 20 63. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-
21 Base platform supports systematic causal inference across the human phenome. *eLife*.
22 2018;7:e34408. doi: 10.7554/eLife.34408.
- 23 64. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of
24 body mass index yield new insights for obesity biology. *Nature*. 2015;518:197. doi:
25 10.1038/nature14177

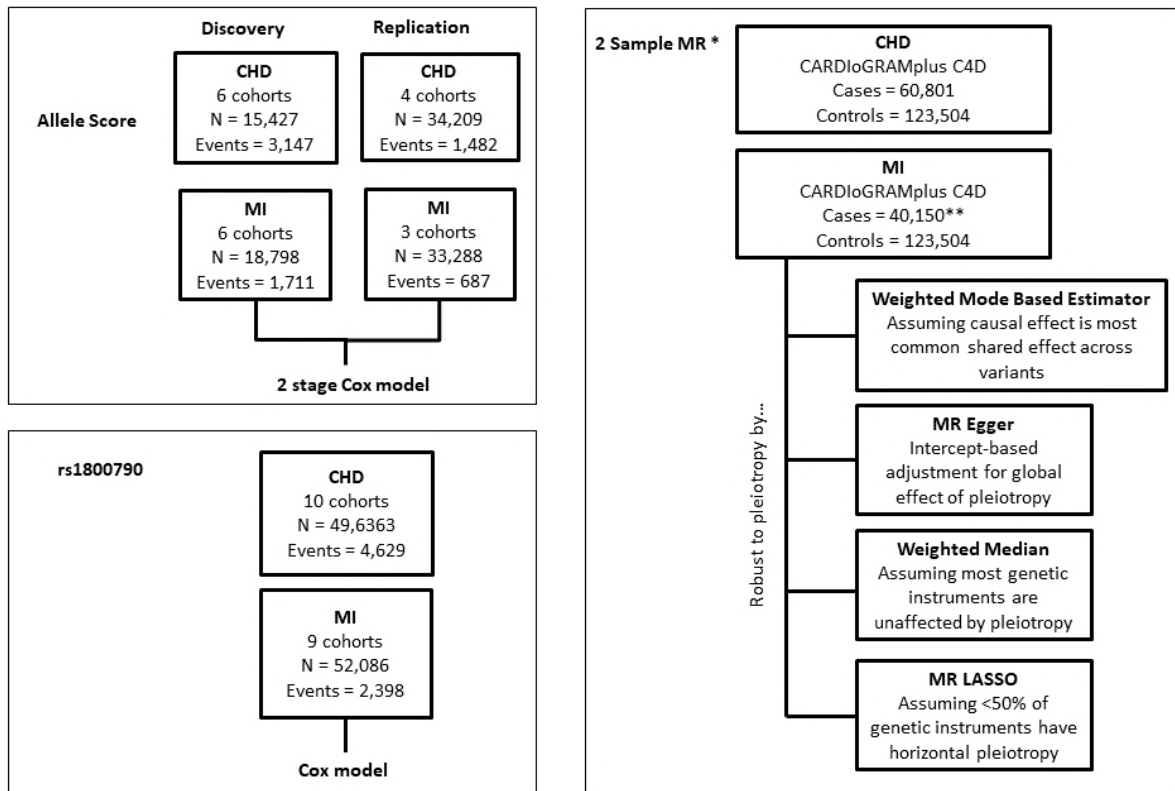
- 1 <https://www.nature.com/articles/nature14177#supplementary-information>.
- 2 65. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Mägi R, et al.
3 New genetic loci link adipose and insulin biology to body fat distribution. *Nature*.
4 2015;518:187. doi: 10.1038/nature14132
- 5 <https://www.nature.com/articles/nature14132#supplementary-information>.
- 6 66. Global Lipids Genetics Consortium. Discovery and refinement of loci associated with
7 lipid levels. *Nature Genetics*. 2013;45:1274. doi: 10.1038/ng.2797
- 8 <https://www.nature.com/articles/ng.2797#supplementary-information>.
- 9 67. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New
10 genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk.
11 *Nature Genetics*. 2010;42:105. doi: 10.1038/ng.520
- 12 <https://www.nature.com/articles/ng.520#supplementary-information>.
- 13 68. Wood AR, Tyrrell J, Beaumont R, Jones SE, Tuke MA, Ruth KS, et al. Variants in the
14 FTO and CDKAL1 loci have recessive effects on risk of obesity and type 2 diabetes,
15 respectively. *Diabetologia*. 2016;59(6):1214-21. doi: 10.1007/s00125-016-3908-5.
- 16 69. R Core Team. R: Language and Environment for Statistical Computing. In:
17 Computing RfS, editor. Vienna, Austria: R Foundation for Statistical Computing; 2015.
- 18 70. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat*
19 *Softw*. 2010;36(3):1-48.
- 20 71. Therneau TM, Lumley T. Package ‘survival’. Verze; 2016.

21

1

2 **Figure 1. Study Outline**

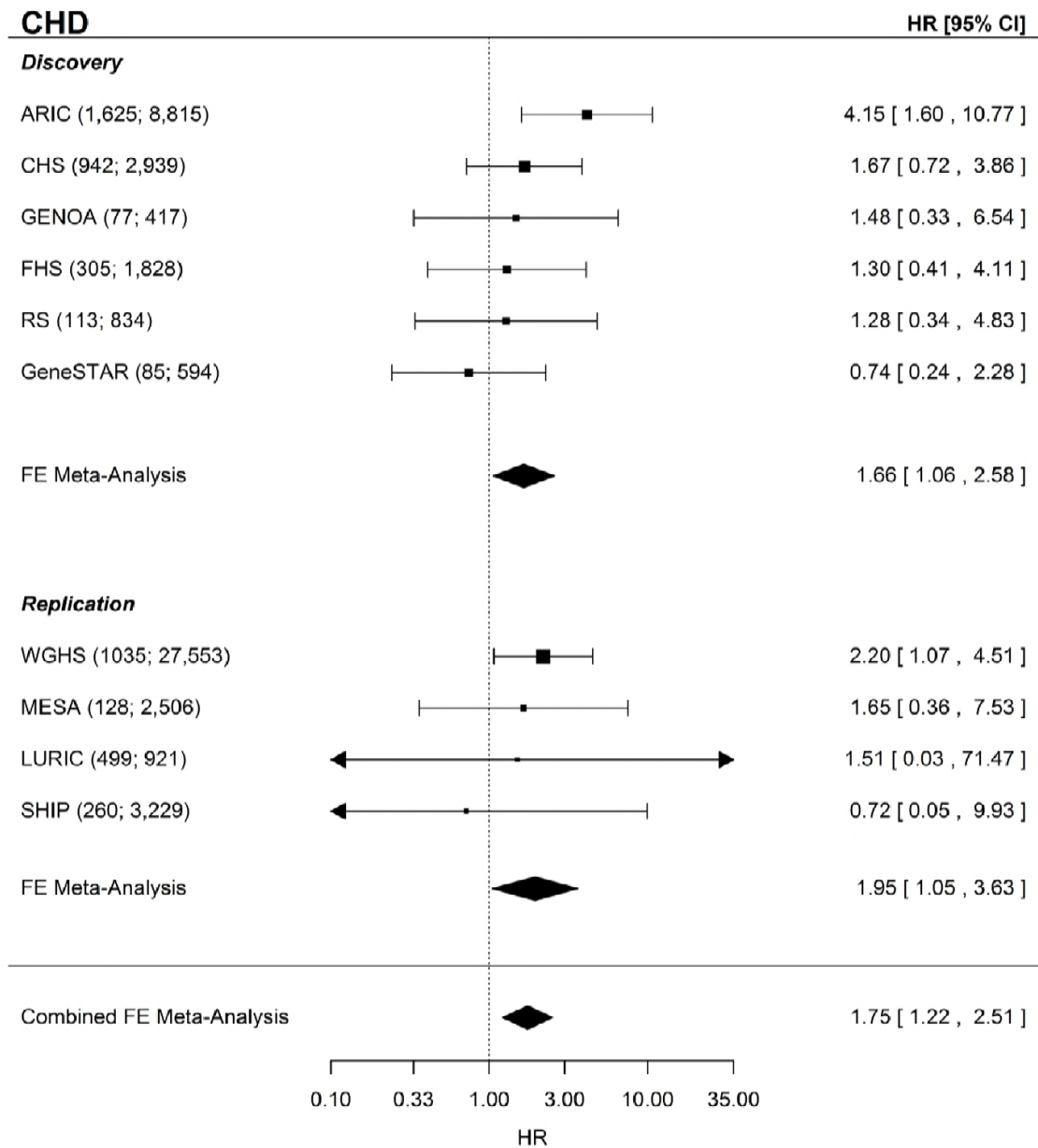
3 Outline of analyses using the allele score, rs1800790 and 2 Sample MR approaches including
 4 the analytic method used to estimate the causal effect, subject to valid MR assumptions, for
 5 all stages of the analysis. CHD = coronary heart disease; MR = Mendelian Randomization;
 6 MI = myocardial infarction



* Estimates of genetic variant fibrinogen association came from 11 cohorts used in incident analyses

** Cases estimated from the percentage of MI cases from total cases as given in Supplemental Table 1 of [38]

7

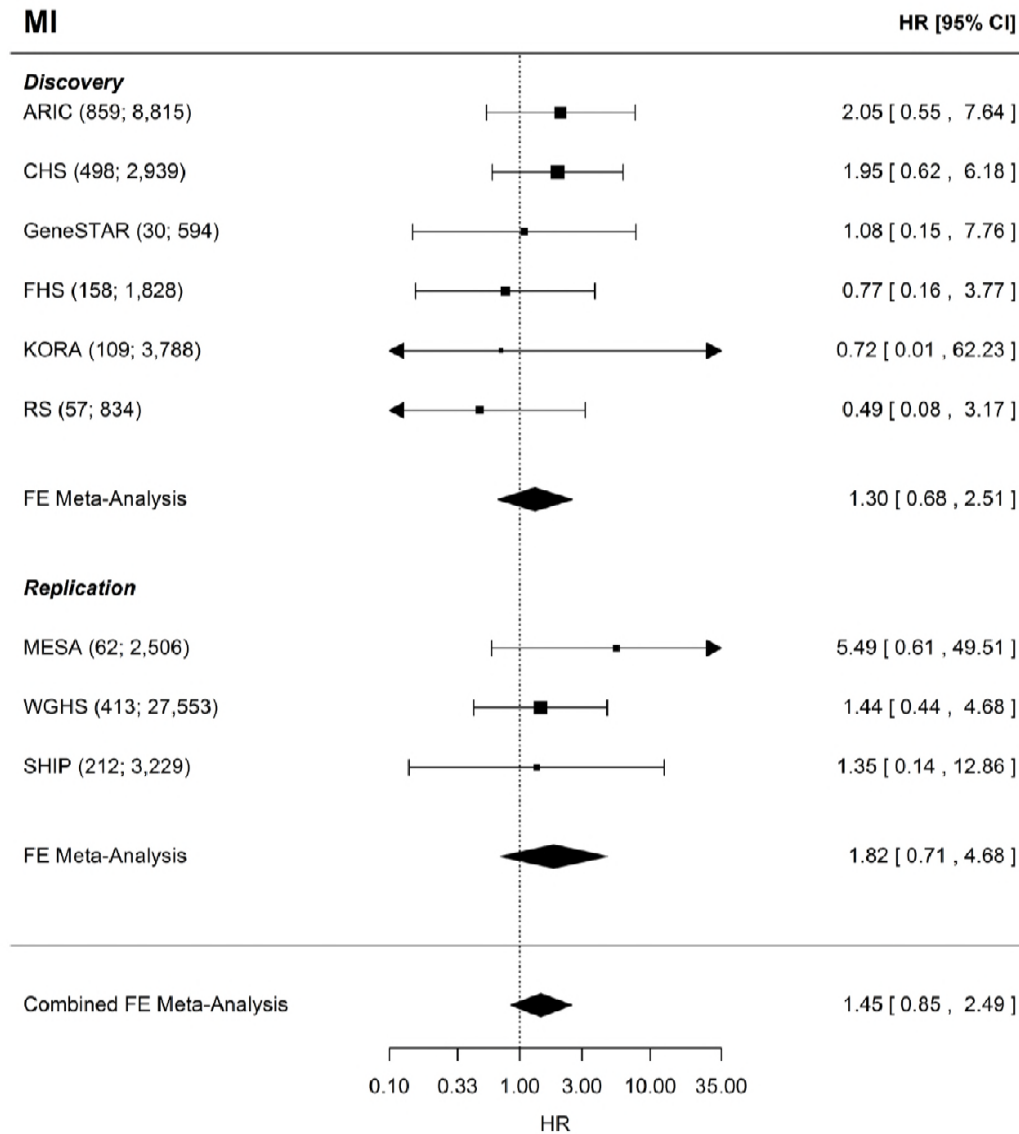


1

2 **Figure 2: CHD Forest Plot**

3 Forest plot of the CHD MR analysis for the discovery, replication, and combined sets of
 4 cohorts. Shown beside each cohort name is the sample size and number of incident CHD
 5 events given as (N events; N total). CHD = coronary heart disease; FE = fixed-effects; HR =
 6 hazard ratio; CI = confidence interval

1



2

3 **Figure 3: MI Forest Plot**

4 Forest plot of the MI MR analysis for the discovery, replication, and combined sets of
 5 cohorts. Shown beside each cohort name is the sample size and number of incident MI events
 6 given as (N events; N total). MI = myocardial infarction, FE = fixed-effects, HR = hazard
 7 ratio, CI = confidence interval

- 1 **Supporting Information Legends**
- 2 Supplemental Methods and Tables.doc
- 3 File containing the Supplemental Online Methods (including cohort specific information) as
- 4 well as the Supplemental Tables (1-5)

	Discovery							Replication			
	KORA	GENOA	ARIC	CHS	GeneSTAR	FHS	RS	MESA	WGHS	LURIC	SHIP
N	3,788	417	8,815	2,939	594	1,828	834	2,506	27,553	921	3,229
Mean (SD)											
MI follow-up time (y)	8.44 (1.52)	-	20.8 (6.03)	10.3 (6.0)	6.72 (3.59)	17.9 (5.32)	9.32 (3.16)	6.25 (1.12)	17.5 (3.76)	-	11.14 (0.8)**
CHD follow-up time (y)	-	10.4 (3.6)	20.0 (6.49)	10.3 (5.9)	6.07 (3.19)	17.9 (5.32)	9.18 (3.24)	6.18 (1.23)	17.4 (3.95)	9.43 (2.55)	11.14 (0.8)**
Age (y)	49.2 (13.9)	58.6 (10.0)	54.2 (5.68)	72.4 (5.4)	51.1 (11.3)	53.8 (10.0)	72.2 (6.83)	62.7 (10.2)	54.6 (7.08)	60.1 (11.5)	46.39 (15.11)
BMI (kg/m ²)	27.2 (4.69)	30.6 (6.3)	27.0 (4.86)	26.2 (4.4)	29.3 (6.3)	27.4 (4.93)	26.7 (3.80)	27.7 (5.10)	25.1 (6.78)	27.5 (4.36)	27.0 (4.75)
Fibrinogen (g/L)	2.63 (0.60)	3.19 (0.80)	2.95 (0.61)	3.14 (0.61)	3.83 (1.08)	3.14 (0.61)	3.95 (0.87)	3.35 (0.70)	3.59 (0.78)	3.69 (0.90)	2.95 (0.68)
log(Fibrinogen)	0.94 (0.22)	1.11 (0.36)	3.97 (0.11)	1.13 (0.19)	1.30 (0.27)	1.12 (0.19)	1.35 (0.21)	1.19 (0.20)	1.25 (0.22)	1.28 (0.26)	1.06 (0.22)
HDL (mg/dL)	57.9 (17.0)	52.5 (14.3)	51.1 (16.7)	55.8 (15.9)	52.1 (15.8)	51.3 (15.3)	204 (24.5)	52.4 (15.7)	53.1 (16.6)	41.8 (11.7)	57.04 (17.1)
LDL (mg/dL)	137 (41.4)	117 (31.0)	137 (37.6)	134 (35.8)	130 (41.6)	126 (33.0)	104 (12.5)	117 (30.3)	122 (37.3)	121 (33.7)	137.3 (44.2)
N (%)											
MI	109 (2.88)	-	859 (9.74)	498 (16.9)	30 (5.05)	158 (8.64)	57 (6.83)	62 (2.50)	413 (1.50)	-	212 (6.5)
CHD	-	77 (18.5)	1,625 (18.4)	942 (32.1)	85 (14.31)	305 (16.7)	113 (13.6)	128 (5.10)	1,035 (3.76)	59 (6.41)	260 (8.0)
Sex (male)	1,854 (48.9)	156 (37.4)	3,983 (45.2)	1,792 (61.0)	321 (54.0)	829 (45.0)	421 (50.5)	1,308 (52.2)	0 (0.00)	499 (54.2)	1,537 (47.6)
Current Smokers	962 (25.4)	47 (11.3)	2,633 (29.9)	322 (11.0)	135 (22.8)	341 (18.7)	144 (17.3)	286 (11.4)	3200 (11.6)	197 (21.4)	1,096 (33.9)
Former Smokers	1,262 (33.3)	157 (37.6)	2,050 (23.3)	1,216 (41.4)	202 (34.0)	404*	429 (51.4)	1,109 (44.4)	10,096 (36.6)	273 (29.6)	1,004 (31.1)
Never Smokers	1,560 (41.2)	213 (51.0)	4,109 (46.6)	1401 (47.7)	257 (43.3)	911*	261 (31.3)	1,104 (44.2)	14,233 (51.7)	451 (49.0)	1,129 (35.0)
Hypertension	1,079 (28.5)	310 (74.3)	2,272 (25.8)	1549 (52.7)	261 (43.94)	563 (30.8)	204 (24.5)	1,097 (43.8)	6,654 (24.2)	625 (67.5)	671 (20.8)
Type 2 Diabetes	565 (14.9)	52 (12.5)	1,659 (18.8)	349 (11.9)	59 (9.93)	129 (7.10)	104 (12.5)	150 (6.70)	0 (0.00)	283 (30.7)	174 (5.4)

Table 1. Clinical Covariates

Clinical covariates for all participating cohorts. KORA did not have incident CHD data and thus did not participate in these analyses. GENOA and LURIC had too few incident MI cases for analysis. * For FHS 172 individuals were not current smokers but were not distinguished as former vs

never smokers thus percentages were not computed for these categories and the N for those with information is given. ** For SHIP only interval censored data was available. Follow-up time represents the time from initial exam to final exam. BMI = body mass index; CHD = coronary heart disease; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; MI = myocardial infarction; NA = not available

Table 2. Multi-variant, Pleiotropy Robust MR Methods

To further examine potential effects of pleiotropy we ran several multi-variant, pleiotropy robust models including MR Egger, Weighted Mode Based Estimator (MBE), Weighted Median, and MR PRESSO. Each uses a different means to account for pleiotropy and has different assumptions used to estimate the causal effect in the presence of pleiotropy. Odds ratios are per 1 g/L increase in genetically determined fibrinogen. CHD = coronary heart disease; CI = confidence interval; MI = myocardial infarction; MR = mendelian randomization

Method	Robust to pleiotropy by ...	CHD Causal OR (95% CI)	MI Causal OR (95% CI)
MR Egger	Intercept-based adjustment for global effect of pleiotropy	0.98 (0.70, 1.39)	0.89 (0.63, 1.26)
Weighted MBE ($\phi = 1$)	Assuming causal effect is most common shared effect across variants	1.09 (0.89, 1.33)	0.98 (0.79, 1.21)
Weighted Median	Assuming most ($\geq 50\%$) genetic instruments are unaffected by pleiotropy	1.12 (0.91, 1.37)	1.03 (0.82, 1.29)
MR PRESSO	Assuming $<50\%$ of genetic instruments have horizontal pleiotropy	1.18 (0.98, 1.42)	1.17 (0.98, 1.40)