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# Causal effects of cardiovascular risk factors on onset of major age-related diseases: A time-to-event Mendelian randomization study

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ABSTRACT

*Backgrounds:* Elucidating the causal effects of common intermediate risk factors on the onset of age-related diseases is indispensable for developing prevention and intervention procedures.

*Methods:* We conducted two-stage time-to-event Mendelian randomization meta-analyses combining five large-scale longitudinal cohorts to investigate dynamic causal effects of cardiovascular disease risk factors including body mass index (BMI), systolic blood pressure (SBP), and lipids on the age-at-onset of age-related diseases. We constructed weighted polygenic scores based on genetic markers from previously reported genome-wide association studies as instrumental variables to estimate the causal effects. To avoid false positive due to potential pleiotropic effects of the genetic markers, we performed a leave-one-out sensitivity analysis and an MR-Egger sensitivity analysis that we expanded in the survival context.

*Results:* Our results show that elevated BMI increases the absolute risk of type 2 diabetes (T2D) ($p = 7.68e-04$), heart failure ($p = 9.03e-03$), and cardiovascular diseases (CVD) ($p = 1.69e-03$) and the causal effects start at different ages. A significant association between BMI and the risk of stroke is observed; however, the sensitivity analyses suggest that the association is attributed to the potential pleiotropic effects of rs2867125 and rs1558902. Raised SBP levels are significantly associated with the development of atrial fibrillation ($p = 6.42e-03$). Low-density lipoprotein cholesterol (LDL-C) levels are inversely associated with the age-at-onset of T2D ($p = 1.05e-02$). In addition, LDL-C and triglycerides are inversely associated with the risks of cancer and T2D, respectively. Nevertheless, the sensitivity analyses suggest that these associations are probably due to pleiotropic effects of several single-nucleotide polymorphisms including rs4970834 and rs1260326.

*Conclusions:* Our results highlight the involvement of BMI in the development of multiple age-related diseases. Some observed causal associations can attribute to pleiotropic effects of some genetic variations. These findings have important implications in unraveling causal effects of common risk factors on age-related diseases and guiding effective intervention strategies to reduce the incidence of these diseases.

## 1. Introduction

Common aging-associated diseases including cancer, various cardiovascular diseases (CVDs), type 2 diabetes (T2D) and neurodegenerative diseases (NDs) such as Alzheimer's disease (AD) are the leading causes of death and major contributors to morbidity, disability, and mortality at old age (Sahyoun et al., 2001). Evidence has shown that most of these diseases are correlated with certain common intermediate risk factors such as body mass index (BMI), blood pressure (BP) and lipids. A fundamental hypothesis in gerontology is that the biological aging process that leads to physical dysfunction and deviation from normal physiological indices and levels of some crucial biomarkers would be implicated in the onset and progression of multiple diseases (Arbeev et al., 2011; Kaeberlein et al., 2015). Nevertheless, it is still elusive whether the deviation from the optimal values of the biomarkers directly results in onset of these diseases or they are concurrent ramifications of certain more fundamental molecular mechanisms underlying the biological aging process, i.e., pleiotropy. Thus, elucidating the potential causal relationship between the intermediate risk factors and the onset of the age-related diseases can contribute to the development of effective intervention procedure and management policy that may lead to remarkable improvement of human healthspan and lifespan. It can also provide more insights into the underlying biological implications in the aging process and pinpointing potential

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physiological determinants of these diseases.

Despite the evidence of association, it is still unclear whether some of these biomarkers play a causal role in the etiology of these diseases and, more interestingly, how the causal effects vary with ageing if they exist. This is because inferring causation from general association analyses is often not straightforward as it is complicated by potentially unmeasured confounders and reverse causality (Pearl, 2000). One of the feasible approaches for causal inference is to construct an instrumental variable (IV) under a parametric model. With the advent of sequencing and microarray techniques in molecular biology, causal effects can be evaluated with the Mendelian randomization (MR) analyses that leverage a large number of identified genetic variants from genome-wide association analyses (GWAS) as IVs (Davey Smith and Hemani, 2014; Didelez and Sheehan, 2007). Previous MR studies based on cross-sectional or longitudinal datasets have reported that BMI, BP and lipids may have causal effects on the incidence of multiple age-related diseases (Hägg et al., 2015; Holmes et al., 2014, 2015; Østergaard et al., 2015; Proitsi et al., 2014). However, to our knowledge, few MR studies have focused on the dynamic causal effects on the age-at-onset of these diseases.

Most recently, several methods using IVs based on the additive hazards model (Aalen, 1978) have been proposed (Li et al., 2015; Tchetgen Tchetgen et al., 2015), which are characterized by straightforward estimation of the dynamic effects over age and more intuitive interpretation of the estimated parameters. MR using the additive hazards model enjoys robustness due to collapsibility compared to proportional hazards model (Martinussen and Vansteelandt, 2013). Compared to the MR analyses in cross-sectional studies, MR analyses with time-to-event outcomes leverage more information from the longitudinal data, so it can provide an estimate of time-varying causal effects on the risk of diseases. The time-to-event MR analysis gives more information about the age at which the effects start and how long they persist. This is of importance because in many cases intermediate risk factors are measured at different ages across the sample at the baseline. In the survival context using the additive hazards model, the assumption of a constant causal effect at different ages, which is not always realistic, can be relaxed (Tchetgen Tchetgen et al., 2015). For example, large BMI at early stage of life may have different effects on T2D compared to middle age. Therefore, it is of enormous interest to investigate how the causal effects are changed and modulated by other environmental factors during the life course.

In this work, we perform time-to-event MR analyses to investigate the causal effects of five cardiovascular disease risk factors including BMI, systolic blood pressure (SBP), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides on eight age-related diseases including T2D, coronary heart disease (CHD), heart failure (HF), myocardial infarction (MI), stroke, cancer, atrial fibrillation (AF) and AD. These diseases are major causes of death in developed societies. We include > 30,000 individuals from five NIH-funded large-scale longitudinal cohorts (Atherosclerosis Risk in Communities study (ARIC), the Framingham Heart Study (FHS), the Multi-Ethnic Study of Atherosclerosis (MESA), the Cardiovascular Health Study (CHS), and the Women's Health Initiative (WHI)). We perform a meta-analysis to combine the causal effects from each cohort, which we estimate based on an additive hazards model. We construct polygenic scores as IVs based on well recognized genetic loci with known mechanisms. The MR method based on polygenic scores has been shown to avoid weak instrument bias (Burgess and Thompson, 2013). As some factors such as pleiotropy and population stratification can violate assumptions in the MR analysis (Burgess et al., 2015, 2016; Taylor et al., 2014), we conduct various sensitivity analyses to minimize the possibility of reporting false positives.

## 2. Material and methods

### 2.1. Study cohorts

We collected datasets from five large-scale longitudinal cohorts including ARIC, FHS, MESA, CHS, and WHI (total 30,505 individuals included) (Table 1). In each cohort, we only included non-Hispanic Caucasian subjects. For FHS, we only included the datasets of the FHS Original cohort (FHS cohort 1) and the FHS Offspring cohort (FHS cohort 2) because the third generation was too young to observe sufficient informative outcomes. Details of the study design, collection of samples, measurement of risk factors and diagnosis of diseases have been described in previous publications (ARIC: (Sharrett, 1992; The ARIC investigators, 1989), FHS: (Cupples et al., 2009; Govindaraju et al., 2008; Splansky et al., 2007), MESA: (Bild et al., 2002), CHS: (Gottdiener et al., 2000), WHI: (The Women's Health Initiative Study Group, 1998)). Table 1 summarizes the basic characteristics of the samples, risk factors, covariates and diseases we investigated.

### 2.2. Phenotypes and covariates

In each cohort, we used the measurements of BMI, SBP, HDL-C, LDL-C and triglycerides at the entry of enrollment, so that the precedence of the factors to the diagnosis of diseases was guaranteed to minimize the possibility of reverse causation. For the disease outcomes, we included T2D, CHD, HF, MI, stroke and CVD in all five cohorts. The CVD variables were constructed separately in each cohort from CHD, HF, MI and stroke to harmonize the definition across the cohorts (The details of the construction of the CVD variable are provided in Table S9). The age at onset of T2D in WHI was reckoned from self-reported treatment history or the age at diagnosis that was categorized into age groups. We included cancer in ARIC, FHS, CHS and WHI, which was not included in MESA due to missing information on age at onset in the original data. In FHS and WHI, we used the cancer variable that excluded skin cancer because the information was either not available or inaccurate. Additionally, we included AF in ARIC and WHI, and AD in FHS and CHS in which we selected AD or mild dementia. In the sensitivity analyses adjusted for potential confounders (described later), we included sex, birth cohort, which were non-heritable variables, and further education

**Table 1**
The basic characteristics of the cohorts included in the MR analyses. Sample size: the total number including non-Caucasian whites. Diseases: the age-related diseases examined in each cohort. AF is only available in ARIC and WHI. AD is only available in FHS and CHS. Cancer is not included in MESA because its age-at-onset information is missing in the original data. Covariate: the covariates adjusted in the main MR analysis. 'Cohort' in FHS is the indicator for generation, i.e., FHS or FHSO. Other confounders: the covariates adjusted in the follow-up sensitivity analyses. BC: birth cohort. SM: smoking history (current smoker, ex-smoker, non-smoker). DR: alcohol consumption history (the concrete definition varies among the cohorts). EDU, education level (in FHS and CHS, the binary variable indicating low education level is used due to large missing data in education level).

| Study | Sample size | Number of families | Male (%) | Average age at baseline ( ± sd) | Diseases | Covariate | Other confounder |
|---|---|---|---|---|---|---|---|
| ARIC | 9810 | 9105 | 47.13 | 54.33 (5.67) | T2D, CHD, HF, MI, AF, cancer, stroke, CVD | Site | BC, Sex, SM, DR, EDU |
| FHS | 4700 | 1482 | 45.28 | 35.91 (9.25) | T2D, CHD, HF, MI, cancer, stroke, AD, CVD | Cohort | BC, Sex, SM, DR, EDU |
| CHS | 3310 | 3310 | 39.76 | 72.41 (5.41) | T2D, CHD, HF, MI, cancer, stroke, AD, CVD | – | BC, Sex, SM, DR, EDU |
| MESA | 2685 | 2649 | 47.86 | 62.74 (10.16) | T2D, CHD, HF, MI, stroke, CVD | Site | BC, Sex, SM, EDU |
| WHI | 10,000 | 9990 | 0 | 67.05 (6.45) | T2D, CHD, HF, MI, AF, cancer, stroke, CVD | Region | BC, SM, DR, EDU |

level, smoking and drinking status as covariates. In CHS and FHS, we determined a smoker and a drinker at the baseline according to cigarettes per day (> 0) and alcohol consumption (> 0), respectively. In CHS and FHS, we used an indicator of low education as there were large missing data in the variable of education level. The inclusion of diseases, covariates and confounders in each cohort is summarized in Table 1.

### 2.3. Accession numbers

This manuscript was prepared using a limited access datasets obtained though dbGaP (accession numbers phs000007.v22.p8, phs000280.v2.p1, phs000209.v12.p3, phs000287.v3.p1).

### 2.4. Genotyping, quality control and imputation

Genotyping of 12,771 ARIC participants (N = 9633 whites, N = 9618 included) and 8224 MESA participants (N = 2685 whites, N = 2455 included) was conducted using an Affymetrix 6.0 array (1000 K SNPs). Genotyping of 9167 participants (N = 4594 included) in FHS was conducted using an Affymetrix 500 K array. Genotyping of 3043 participants in CHS was done using an Illumina Human Omni1-Quad array. In WHI, genotyping were performed in two separate groups that used an Illumina Human Omni1-Qud array (1 M SNPs) and an Illumina Human Omni Express array (~730 K SNPs). All genotyped SNPs were further filtered for the analysis according to the exclusion criteria of the Hardy-Weinberg equilibrium test $p < 1e-05$ and missing rate < 5%. We imputed the missing genotypes for the SNPs that were used in the construction of the polygenic scores for the MR analyses (details provided in the following subsection). The imputation was performed using IMPUTE2 (Howie et al., 2009) with a 1000 Genomes Project Phase I reference panel and those SNPs with an imputation information score < 0.8 were excluded. The imputation information score and minor allele frequency (MAF) for each SNP in each cohort is provided in the Supplementary materials Tables S1–S5.

### 2.5. Construction of polygenic scores

We created weighted genetic scores based on a group of selected independent genetic variants that have been recognized to show biological link to each risk factor. Following the same strategy as previous studies (Østergaard et al., 2015; Richmond et al., 2014) for including associated variants, we adopted 32 variants and 25 variants with their effect sizes estimated from large-scale GWAS meta-analyses (Speliotes et al., 2010; Studies, 2011) for BMI and SBP, respectively. For lipids, we included the SNPs that were reported in a large-scale meta-analysis of blood lipid traits (Voight et al., 2012) and were carefully selected in a recent MR study (Holmes et al., 2015). These included 48 variants for HDL-C, 42 variants for LDL-C and 67 variants for triglycerides. The lists of the variants used for construction of the polygenic scores in each cohort are provided in Supplementary materials Tables S1–S5. Denote $\beta_j$ the effect size estimated from the meta-analyses for a SNP $j$, and $g_{ij}$ its genotype (either genotyped or imputed) for an individual $i$ assuming an additive genetic model. The polygenic score $G_i$ for a specific risk factor with $J$ associated variants for individual $i$ was calculated according to.

$$G_i = \sum_{j=1}^{J} \beta_j \cdot g_{ij}.$$

### 2.6. Time-to-event MR analysis

For the time-to-event MR analyses, we adopted a two-stage regression approach utilizing an additive hazards model proposed by (Tchetgen Tchetgen et al., 2015). Specifically, we first fitted the following linear regression model for an intermediate risk factor $M$.

$$M = c_0 + c_1 \cdot G + c_2 \cdot X + \varepsilon,$$

in which $\varepsilon$ is the random error term following a zero–mean normal distribution and $X$ stands for other observed covariates such as cohorts and regions that are assumed to be independent of the polygenic score $G$. Thereby we obtained a predicted mean of the risk factor $\widehat{M} = \widehat{c_0} + \widehat{c_1} \cdot G + \widehat{c_2} \cdot X$. In the second stage, given the age at an event $T^* = \min(T, C)$, where $T$ is the failure time and $C$ is the assumed censoring time that is independent of the failure time, and the variable $\delta \in \{0, 1\}$ indicating $T^* = T$ or $C$, we fitted the following additive hazards model

$$h(t \mid G, X) = b_0(t) + b_1(t) \cdot \widehat{M} + b_2(t) \cdot X,$$

in which $h(t \mid G, X)$ is the conditional hazard function evaluated at $t$ given only $G$ and $X$, $b_0(t)$ is the baseline hazard, $b_1(t)$ is the time-varying causal effect of the risk factor $\widehat{M}$ (Thus, $B_1(s) = \int_0^s b_1(t)dt$ is the cumulative causal effect up to the time $s$) and $b_2(t)$ is the effect of the covariates. Following the similar spirit of (Lamarca et al., 1998), we adopted age as the time scale because age per se was of primary interest and could be a potential confounder. We used the age at entry into the study as left censoring. The effect size $b_1(t)$ of our interest is interpreted as the change of the absolute hazard with respect to one unit change of $\widehat{M}$. In our main MR analyses, we fitted a restricted model in which we assumed an age-invariant causal effect (i.e., $b_1(t) = b_1$), and then relaxed this assumption for significant findings ($p < 0.05$) in the follow-up analyses in pursuit of its dynamic effects. The estimate of the effect size $\widehat{b_1}(t)$ was computed using the R package *timereg* (Martinussen and Scheike, 2007). The package also provides a Kolmogorov–Smirnov test for the hypothesis of a constant effect ($b_1(t) = b_1$). As pointed out previously (Tchetgen Tchetgen et al., 2015), the standard error of $\widehat{b_1}(t)$ should take into account both the uncertainty of the estimation in the first stage and the family structure in some studies such as FHS. We estimated the standard error using a non-parametric cluster bootstrap method (Field and Welsh, 2007) with 500 bootstrap samples that were drawn based on the family structure. In the analysis assuming a constant effect size, we conducted a meta-analysis based on a fixed-effects model combining the estimates from the five cohorts. More specifically, we calculated the combined causal effect size as $\widehat{b_1^M} = \sum_k \widehat{b_1^k} w_k / \sum_k w_k$ and its standard error as $sd(\widehat{b_1^M}) = 1/\sqrt{\sum_k w_k}$, in which $k$ is the index of the studies and $w_k = 1/var(\widehat{b_1^k})$ is the weight for the study $k$. The overall strategy of the MR analysis is depicted in Fig. 1.

### 2.7. Sensitivity analysis

We performed several sensitivity analyses to assess the robustness of the identified causal effects and ruled out potential false positives. To examine whether and the extent to which the findings were affected by other potential confounders that were not included in the main analyses, we performed a sensitivity analysis further including birth cohort, sex, education level, smoking and alcohol consumption history up to the enrollment as covariates. This is particularly relevant for the risk factors that e.g., differ in sex as inclusion of these covariates improves the estimate of the effect size of the polygenic score. As the stringency of criteria for the IV assumption might not be maintained for all variants in a polygenic score (Burgess and Thompson, 2013), we used an MR-Egger method (Bowden et al., 2015) as a sensitivity analysis to examine the influence of potential pleiotropic effects of the SNPs in the polygenic scores. MR-Egger gives a consistent estimate of the causal effect if a weaker assumption (the instrument strength independent of direct effect assumption) is satisfied. As MR-Egger is originally proposed for a linear model (Bowden et al., 2015), we extended it for the additive hazards model (The detailed derivation is given in the supplementary materials Text S1). We further conducted a leave-one-out

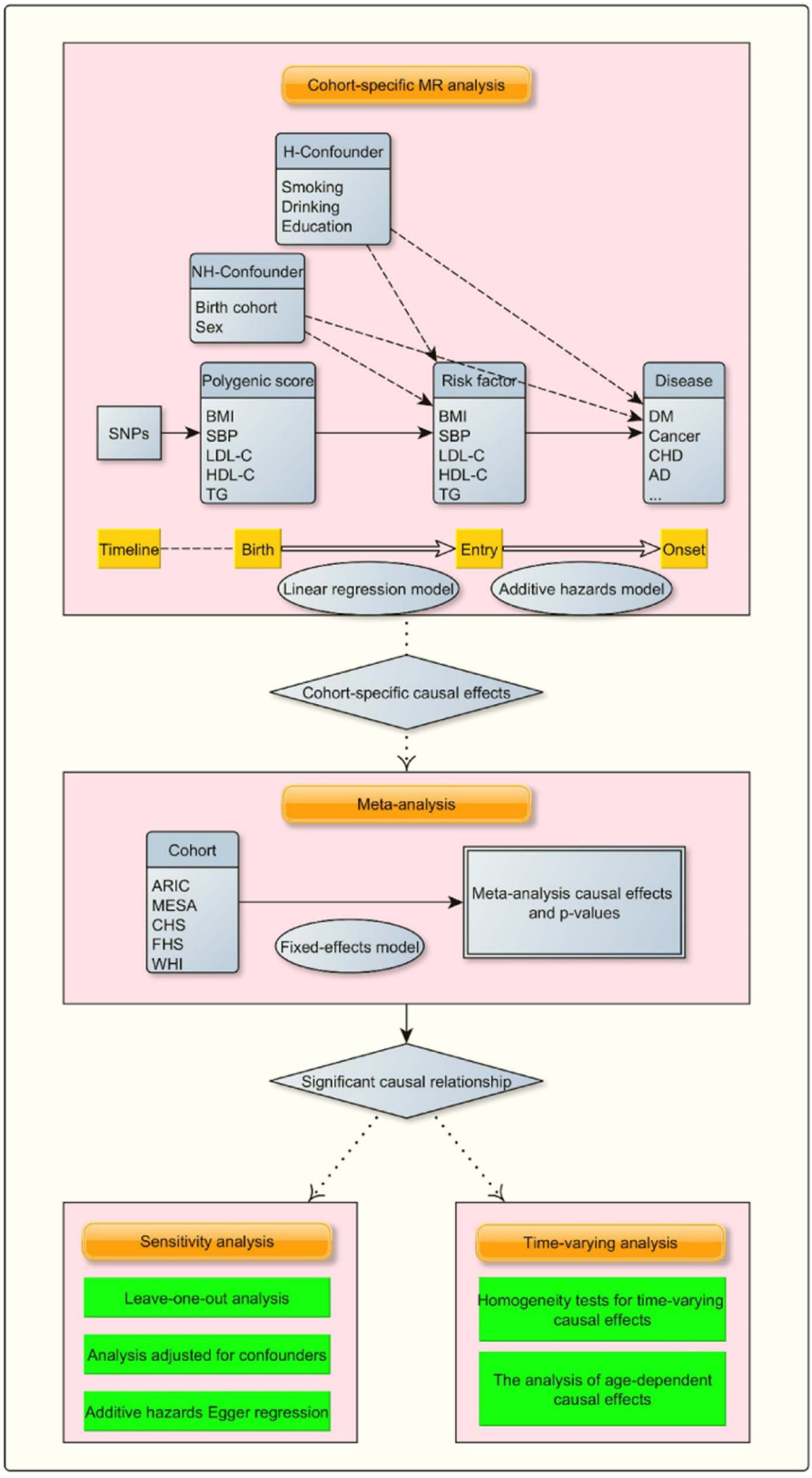


**Fig. 1.** A flowchart of the time-to-event MR meta-analyses. H-confounder: heritable confounders. NH-confounder: non-heritable confounders. Entry: the age at the entry into the follow-up cohort study. Significant causal relationship: the associations with a p-value $< 0.05$.

sensitivity analysis for the identified associations to check that the causal effect was not driven by a specific variant, in which the MR analysis was repeated with each SNP removed from the polygenic score. This analysis could discover those SNPs that harbor independent pleiotropic effects on both the intermediate risk factor and the disease. We also performed a meta-analysis for T2D without WHI because the estimated age at onset of T2D was less reliable in WHI. Another sensitivity analysis was conducted for FHS alone, in which we chose the age at genotyping as the starting point of the follow-up. This removed the potential selection bias for some diseases during the long interval in FHS between the entry into the study and the genotyping, and consequently we did not observe evident difference in the results.

## 3. Results

Each constructed polygenic score was significantly ($p < 0.05$) associated with its risk factor (Table S7) (except for SBP in CHS when adjusted for the covariates and this was due to smaller sample size resulting from exclusion of missing data on the covariates), validating the assumption of the prediction of IVs for the risk factors. Conditional upon the risk factors, the vast majority of the associations between the polygenic scores and the age-at-onset were non-significant (Table S10), indicating no direct evidence of the violation of the conditional independence assumption between most polygenic scores and outcomes (except for HDL-C and T2D, TG and T2D, LDL-C and AD in several studies which will be discussed later separately). Table S10 also shows that the vast majority of the risk factors were significantly associated with the hazards of the diseases. The results from the main MR meta-analyses (without the adjustment for other covariates) are plotted in Fig. 2 (The detailed results are given in Table S12). From the main analyses, we observed 6 significant associations including BMI with T2D, HF, stroke and CVD, SBP with AF, and LDL-C with cancer. Two additional significant associations (triglycerides and T2D, LDL-C and AF) were identified in the sensitivity analyses adjusted for the other covariates (Fig. 3). From the analysis of T2D excluding WHI, we found one more significant association between LDL-C and T2D. For these significant associations, the results from the leave-one-out sensitivity analyses and the MR-Egger analyses, which scrutinized the reliability of the findings, suggested that some of the associations probably were originated from potential pleiotropic effects of certain genetic variants.

### 3.1. Elevated BMI levels associated with development of DM, HF and CVD

From the main MR analyses, we observed that per 1 $kg/m^2$ increase of BMI significantly ($p < 0.05$) causally increased the hazard of T2D by $8.80e-04$ (increasing 0.88 cases per 1000 person-year) (beta $= 8.80e-04$, $p = 7.68e-04$). This causal effect of BMI became more significant after adjusted for the covariates (beta $= 1.04e-03$, $p = 1.49e-04$) (Table S12). The result was consistent with the estimate from MR-Egger, which also suggested no evidence of directional pleiotropy (Table S11). This association remained significant in the analyses without WHI when adjusted for the covariates ($p = 2.28e-2$) (Table 2). The leave-one-out sensitivity analyses suggested that no single SNP in the polygenic score was predominantly responsible for the causal association (Table S8). Most of the diagnosis of T2D occurred within 10 years after the enrollment in ARIC, MESA, CHS and WHI and between 20 and 30 years in FHS (Fig. S1). The increasing effects of BMI on the hazards of T2D were consistent across the five studies (Fig. 4). A steep growth of the dynamic effects estimated from the age-dependent analysis became evident from age 50 and shared similar patterns in ARIC, CHS, FHS and WHI until age 70 (Fig. 5). The test for age-invariant effects suggested that the causal effects of BMI during the follow-up interval did not significantly deviate from a constant in all cohorts except WHI (Table S6).

We found that elevated BMI levels also significantly ($p < 0.05$) increased the hazard of HF (beta $= 4.53e-04$, $p = 9.03e-03$) in the main MR analyses, and this causal association became more significant after the adjustment for the covariates (beta $= 4.92e-04$, $p = 3.90e-03$) (Table S12). The detrimental effects of BMI were observed in all cohorts except MESA (Fig. 4). The result was consistent with the estimate from the MR-Egger analyses (Table S11). The leave-one-out sensitivity analyses suggested that no single SNP in the polygenic score dominated the causal association (Table S8). Similar to T2D, the dynamic effects shared similar patterns in ARIC, CHS, FHS and WHI (Fig. 5). The test for the age-invariant effects suggested that the effects of BMI during the follow-up interval did not significantly deviate from a constant across all cohorts (Table S6).

We observed that higher BMI levels were associated with the risk of CVD in the main MR analyses (beta $= 1.07e-03$, $p = 1.69e-03$) and the analyses adjusted for the covariates (beta $= 1.01e-03$, $p = 4.67e-03$) (Table S12). The effects on the hazards of CVD were consistent across all of the cohorts (Fig. 4). The result was consistent with the estimate from MR-Egger (Table S11). The age-dependent analyses suggested that the accumulative effects of BMI on the hazards of CVD started to rise immediately after the entry into the follow-up across these cohorts (Fig. 5). We also observed that the effect was age-dependent only in FHS ($p = 9.0e-03$) (Table S6) and dropped dramatically after age ~80.

In addition, we found that elevated BMI levels seemed to be significantly ($p < 0.05$) associated with the increasing hazards of stroke (beta $= 2.83e-04$, $p = 3.78e-02$), and this association remained significant after adjusted for the covariates (beta $= 2.98e-04$, $p = 4.23e-02$) (Table S12). The effects of BMI on the hazards of stroke were consistent across the five cohorts in the main analyses (Fig. 4). Nevertheless, this association became non-significant after removing certain SNPs (e.g., rs2867125 or rs7359397 (located on 16p11.2)) from the polygenic score (Table S8). The result from MR-Egger also showed significant directional pleiotropic effects (Table S11). The test for age-invariant effects suggested that the effects of BMI across the follow-up interval did not significantly deviate from a constant in ARIC, MESA, CHS and WHI (Table S6). The dynamic effects started to rise from age ~65 and shared very similar pattern across the five cohorts (Fig. 3).

### 3.2. Elevated SBP levels associated with development of AF

We observed that elevated SBP levels increased the hazard of AF (beta $= 1.98e-04$, $p = 6.42e-03$), and after adjusted for the covariates, this association remained significant (beta $= 1.83e-04$, $p = 6.19e-03$) (Table S12). The result from MR-Egger showed no sign of significant directional pleiotropic effects (Table S11). There was no single underlying SNP in the polygenic score that drove this association (Table S8). The age-dependent analyses showed that the effects rose dramatically after age ~65 in both cohorts (ARIC and WHI). We did not observe an association between AF and any of the other intermediate risk factors.

### 3.3. LDL-C levels are inversely associated with hazards of cancer and T2D

We observed that LDL-C levels were significantly ($p < 0.05$) inversely associated with the hazards of cancer in the main analyses (beta $= -5.28e-05$, $p = 2.65e-02$). This negative association was replicated after adjusted for the covariates (beta $= -4.78e-05$, $p = 3.57e-02$) (Table S12). The negative effect on the hazards was observed in ARIC, CHS and FHS, but not in WHI (Fig. 4). The age-dependent analyses showed that the dynamic effect estimated from WHI turned into positive and rose steadily after age ~75, which was in an opposite temporal pattern compared to those from the other cohorts (Fig. 6). The onset of cancer occurred on average 5 years since the enrollment in ARIC and CHS, almost a decade in WHI, and ~30 years in FHS (Fig. S1(c)). Although the result from MR-Egger showed no evidence of directional pleiotropic effects (Table S11), the leave-one-out analyses showed that the association was no longer significant after excluding rs4970834 (located on 1p13.3) from the polygenic score ($p = 0.0613$) (Table S8).

We also observed that LDL-C was significantly ($p < 0.05$) inversely associated with the hazards of T2D in the analysis without WHI regardless of the covariates adjusted (beta $= -7.98e-05$, $p = 1.05e-02$ in the main analyses, beta $= -8.14e-05$, $p = 1.42e-02$ after adjusted for all covariates) (Table 2), and the negative effect was consistent in all of the four cohorts (Table S7). The leave-one-out analyses showed that these associations still remained significant, indicating no single underlying SNP drove the associations (Table S8).

### 3.4. Relationship between triglycerides and risk of T2D

We did not observe significant inverse association between triglyceride levels and the hazards of T2D in the main MR analyses; however, the association became significant when adjusted for all covariates (Fig. 3). And it became even more significant in the sensitivity analysis of T2D without WHI regardless of the adjustment of the covariates (Table 2). The inverse association was observed consistently across all cohorts (Fig. 4). The age-dependent analyses suggested that the cumulative negative effects increased immediately after the entry into the enrollment, particularly in ARIC, FHS and MESA, and then leveled off after age ~70 (Fig. 6). However, this association became non-significant after some SNPs (e.g. rs1260326) were removed from the polygenic score (Table S8). In addition, the result from MR-Egger also showed that the association was not significant (Table S11).

### 3.5. No risk factors associated with AD

For AD, we did not observe an association with any of the risk factors except LDL-C, which was significant only in CHS (Table S7); however, the association was no longer significant after excluding rs2075650 or rs10402271. The results from MR-Egger also suggested no evidence of causal effects of BMI, SBP, LDLC and HDLC. For triglyceride, MR-Egger detected a causal effect while directional pleiotropy was also observed.

## 4. Discussion

In this study, we perform time-to-event MR analyses to unravel the causality between five intermediate risk factors and the age-at-onset of age-related diseases. Combining the results from the MR analyses using the elaborately constructed polygenic scores and the follow-up sensitivity analyses, we found evidence for four associations between genetic predicted biomarkers and risk of diseases. By leveraging the additive hazards model, we have a refined picture of the dynamic pattern of causal effects if any. Combining with the MR-Egger and leave-one-out sensitivity analyses, we further found that some significant associations identified from the score-based two stage MR analyses are probably due to pleiotropic effects of certain SNPs.

Specifically, higher levels of BMI are associated with increased hazards of T2D. It is found that BMI as well as obesity is a strong predictor of T2D (Garber, 2012; Meisinger et al., 2006) and, in particularly, is the risk factor with the highest impact in Indian population (He et al., 2015). Recent MR studies with large sample sizes provide more evidence for the causal relationship between BMI and T2D (Corbin et al., 2016; Holmes et al., 2014; Lyall et al., 2017). Our result is in line with the previous studies and further discovers that obesity had nearly constant detrimental effects on the onset of T2D before age of ~70, and after that, the effects from the five cohorts diverged with different temporal patterns.

Besides BMI, numerous studies report that high triglycerides are frequently associated with increased risk of T2D (He et al., 2015; Hjellvik et al., 2012; Tirosh et al., 2008). However, MR studies either do not confirm (De Silva et al., 2011) or even report negative associations between genetic predisposition to elevated triglyceride levels and higher risk of T2D (Klimentidis et al., 2015; White et al., 2016). Although our results adjusted for the covariates show that there is a protective effect of triglyceride on the risk of T2D, both the leave-one-out analysis and the MR-Egger analysis suggest that the association is due to pleiotropy of certain SNPs including rs1260326. Previous reports (Klimentidis et al., 2015; Klimentidis and Arora, 2016) point out the likely pleiotropic nature of triglycerides-related SNPs such as rs1260326 in *GCKR* gene as one of several drivers of the pleiotropic effects. The non-synonymous common variant rs1260326, with inverse effects on triglycerides and insulin resistance, is located in glucokinase regulatory protein (GCKR) that plays a critical role for maintaining glucose homeostasis and has pleiotropic effects on multiple metabolic phenotypes (Brouwers et al., 2015; Raimondo et al., 2015; Van Schaftingen et al., 1994). In addition, rs1260326 is in strong LD with other variants in *GCKR* associated with a range of metabolic phenotypes (Dupuis et al., 2010; Orho-Melander et al., 2008; Sparsø et al., 2008).

Although plasma LDL-C levels are usually normal in T2D patients (Sniderman et al., 2001), an increased proportion of atherogenic small dense LDL particles and modified LDL (oxidized LDL and glycated LDL)

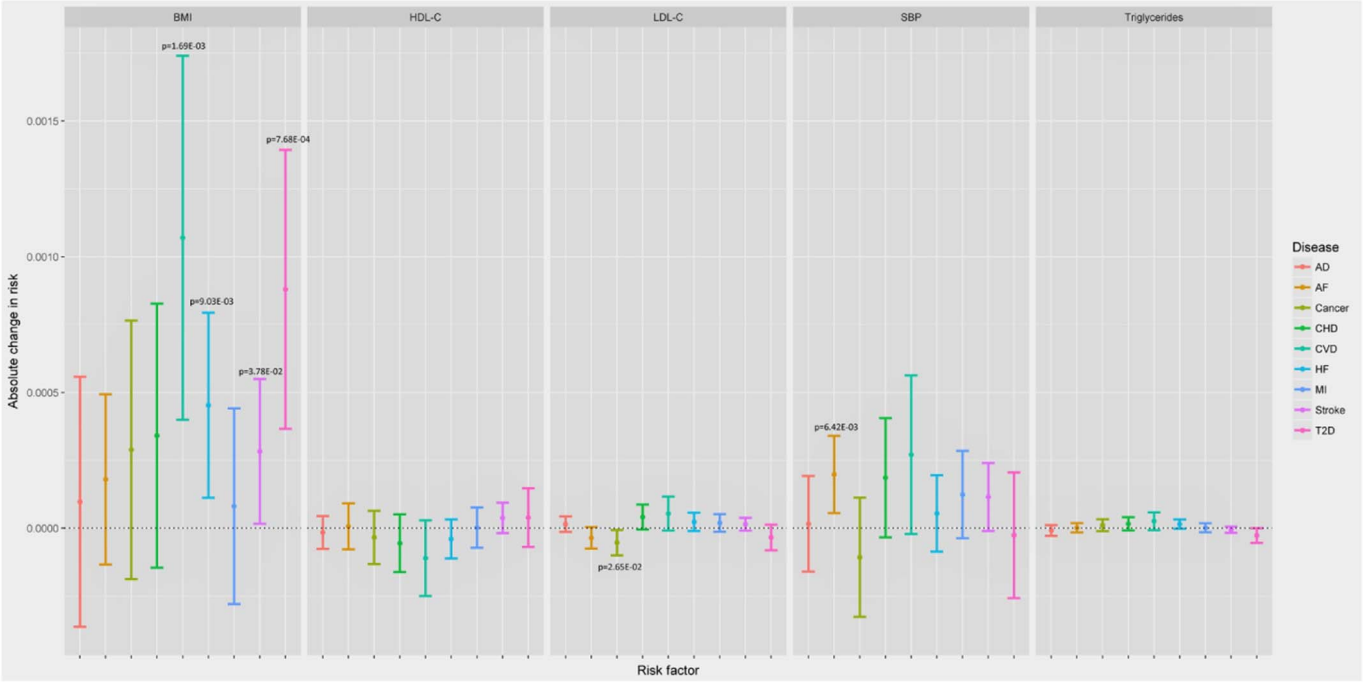


**Fig. 2.** Results from the main meta-analyses for the association between five genetically predicted risk factors and the age-at-onset of nine age-related diseases. The model used in this analysis assumes a constant causal effect over age. The statistically significant associations ($p < 0.05$) are annotated.

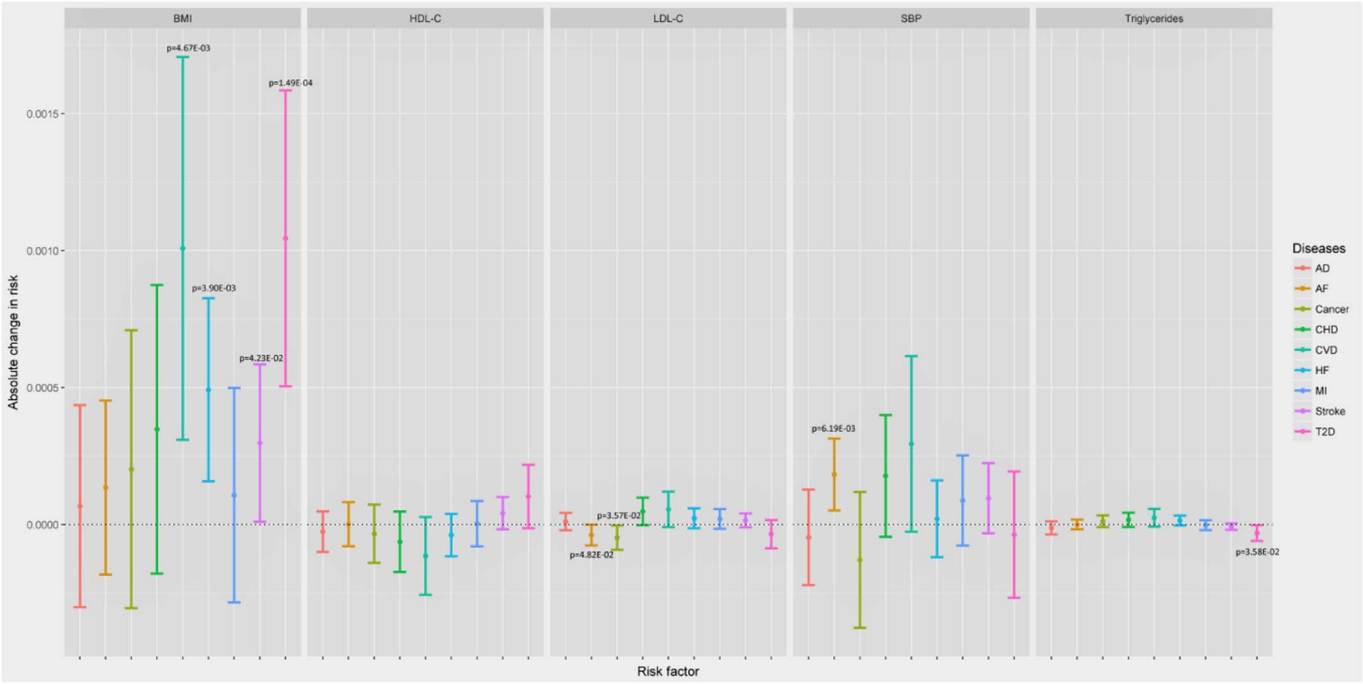


**Fig. 3.** Results from the sensitivity meta-analyses adjusted for sex, birth cohort, education and smoking and alcohol consumption history for the association between five genetically predicted risk factors and the age-at-onset of nine age-related diseases. The model used in this analysis assumes a constant causal effect over age. The statistically significant associations ($p < 0.05$) are annotated.

have been reported (Njajou et al., 2009; Vergès, 2009). Insulin resistance and pancreatic β-cell dysfunction are the core problems of T2D. LDL-C has the adverse effects on insulin secretion and modified LDL (e.g., ox-LDL) has the negative effects on pancreatic β-cell survival. Ox-LDL levels increase in diabetic patients over time independently of maintaining optimized levels of LDL-C (Nakhjavani et al., 2010). Recent MR studies using MR-Egger (Bowden et al., 2015; White et al., 2016) find that increased LDL-C levels are associated with lower risk of T2D, which is consistent with our results.

A very recent large-scale analysis based on the summary statistics from twenty-three prospective studies reports strong association between BMI and HF incidence (Aune et al., 2016), which is in accordance with the results from our analyses. However, it is unclear why a protective effect of BMI was observed on the risk of HF in MESA. Although overweight and obesity are linked to HF (Clark et al., 2014), the nature of this association is not clear. For example, the study of 5881 FHS participants (Kenchaiah et al., 2002) suggests that obesity is an important risk factor for HF. However, the authors observed the smaller effect of BMI on the risk of HF in subjects with hypertension and the lack of the effect of BMI on the risk of HF in subjects with MI, although the latter could be because of the small sample. BMI was not associated with HF risk in (Voulgari et al., 2011). Also, HF patients with increased BMI have reduced mortality rates (Clark et al., 2014; Oreopoulos et al., 2008). The reasons for the obesity paradox in HF and other CVDs remain unclear. Furthermore, the obesity paradox can be sensitive to the cardiorespiratory fitness (Clark et al., 2014; Lavie et al., 2013). The results in MESA may, therefore, be plausibly explained by different degree of physical capacity of the study participants.

In our main analyses, we did not find a significant causal effect of BMI on the risk of CHD although the effect was significant in the sensitivity analyses using MR-Egger. Multiple recent MR studies report large causal effects of BMI on CHD (Dale et al., 2017; Hägg et al., 2015; Lyall et al., 2017). The difference is probably due to the smaller number of the CHD cases in our study, and consequently there is no enough statistical power to detect the effect.

A previous MR study using a logistic model does not find significant association between BMI and the incidence of stroke (Holmes et al., 2014), while a more recent MR study using Cox proportional hazards model suggests a causal effect of adiposity on development of ischemic stroke (Hägg et al., 2015), which is consistent with our results from the main MR analyses. Nevertheless, our results from MR-Egger show the evidence of directional pleiotropy, and the leave-one-out analysis further suggests that rs2867125 and rs7359397 can be the key SNPs underlying the association with stroke. Rs7359397, located in the 16p11.2 region, is in perfect LD with multiple SNPs in the *SH2B1* gene (Jamshidi et al., 2007), including the non-synonymous rs7498665 SNP associated with total fat, waist circumference, body weight and BMI (Speliotes et al., 2010). *SH2B1* adapter protein is involved in multiple signaling

**Table 2**
Results from the sensitivity analysis of association between five genetically predicted risk factors and the age-at-onset of T2D, in which the WHI cohort is excluded. The statistically significant associations ($p < 0.05$) are highlighted in boldface.

| Risk factor | Main analysis | | | Analysis adjusted for all covariates | | |
|---|---|---|---|---|---|---|
| | Change in absolute risk | SD | p | Change in absolute risk | SD | p |
| BMI | 6.70E − 04 | 3.50E − 04 | 5.57E − 02 | **9.07E − 04** | **3.99E − 04** | **2.28E − 02** |
| SBP | − 9.52E − 05 | 1.68E − 04 | 5.71E − 01 | − 1.66E − 04 | 1.96E − 04 | 3.98E − 01 |
| HDL-C | 3.99E − 05 | 6.69E − 05 | 5.51E − 02 | 9.98E − 05 | 7.55E − 05 | 1.86E − 01 |
| LDL-C | **− 7.98E − 05** | **3.12E − 05** | **1.05E − 02** | **− 8.14E − 05** | **3.32E − 05** | **1.42E − 02** |
| Triglycerides | **− 4.78E − 05** | **1.88E − 05** | **1.09E − 02** | **− 5.11E − 05** | **2.05E − 05** | **1.25E − 02** |

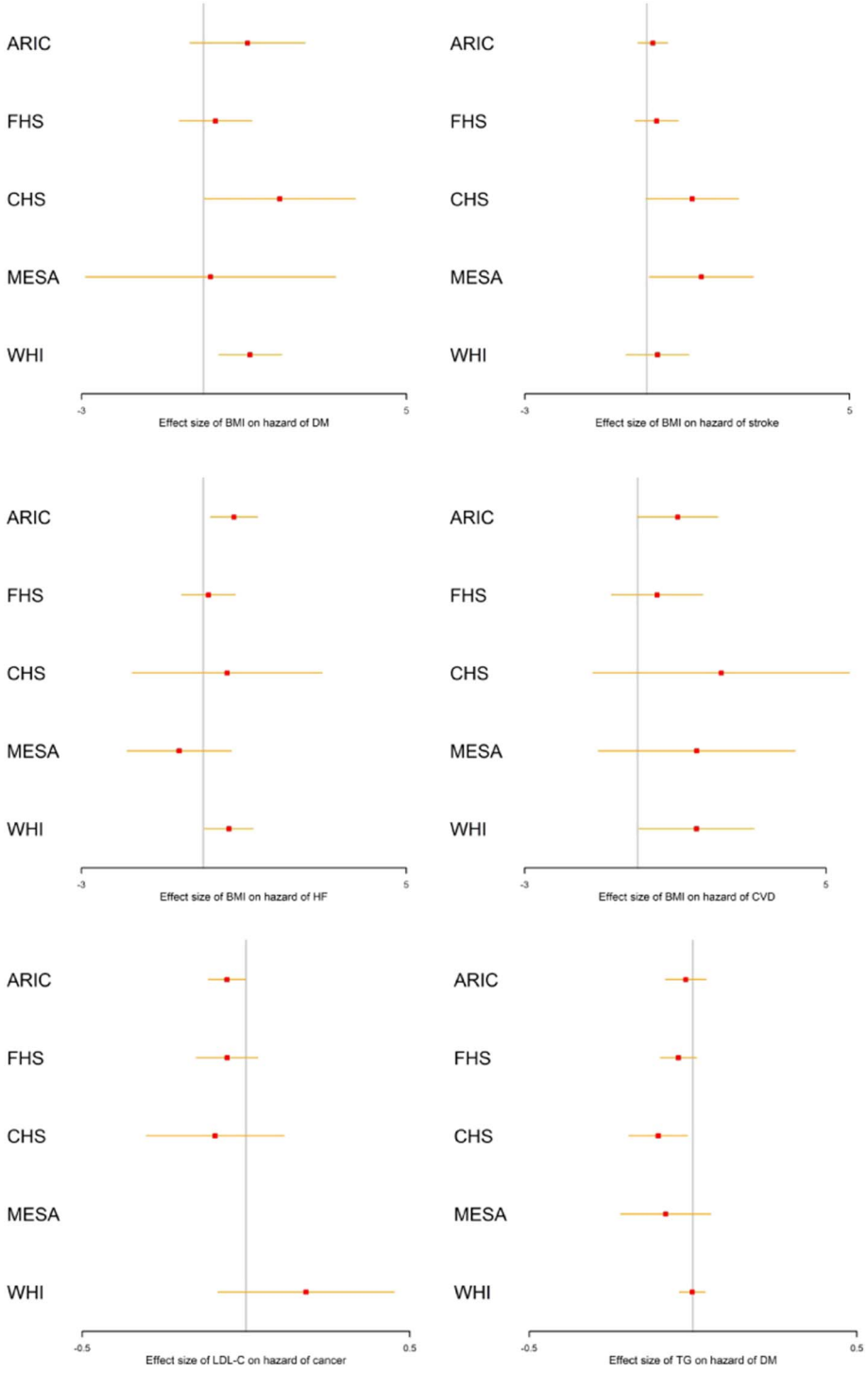


**Fig. 4.** Forest plots of the estimated effect sizes with the 95%CIs in the five cohorts for the six significant associations identified from the main meta-analyses. X-axis: the effect size ($10^{-3}$) in the additive hazards model.

pathways including insulin and leptin signaling (Li et al., 2007, p. 1; Maures et al., 2007, p. 1). The level of the adipose-derived hormone leptin is associated with the risk of stroke (Kim et al., 2012; Liu et al., 2010; Söderberg et al., 2003).

We found that the inverse association between LDL-C levels and the onset of cancer was consistent across three of the cohorts except WHI, in which the effect turns into positive after age ~70. As WHI consists of only female subjects, the positive association was probably due to breast cancer which has the biggest prevalence among all cancers in women. A previous study utilizing in vitro and in vivo models of cholesterol enrichment provides novel mechanistic evidence that high LDL-C levels promote breast cancer progression (Rodrigues dos Santos et al., 2014). Without WHI, our results are in line with a previous study (Lavigne et al., 2012) that assesses the trend of LDL-C for an extended period of time prior to cancer diagnosis without history of lipid-lowering therapy using data from the FHSO. This previous study demonstrates an inverse association between LDL-C and cancer extending over 18 years prior to diagnosis. However, the causal role of LDL-C in the biological etiology of cancer is still elusive. Another MR study suggests that LDL-C levels per se do not cause cancer (Benn et al., 2011), but the non-significant result is probably due to the lack of statistical power as only several SNPs associated with low LDL-C levels are used in this study. One hypothesis for the inverse association is potential reverse causation (Trompet et al., 2009) because cancer may start to develop

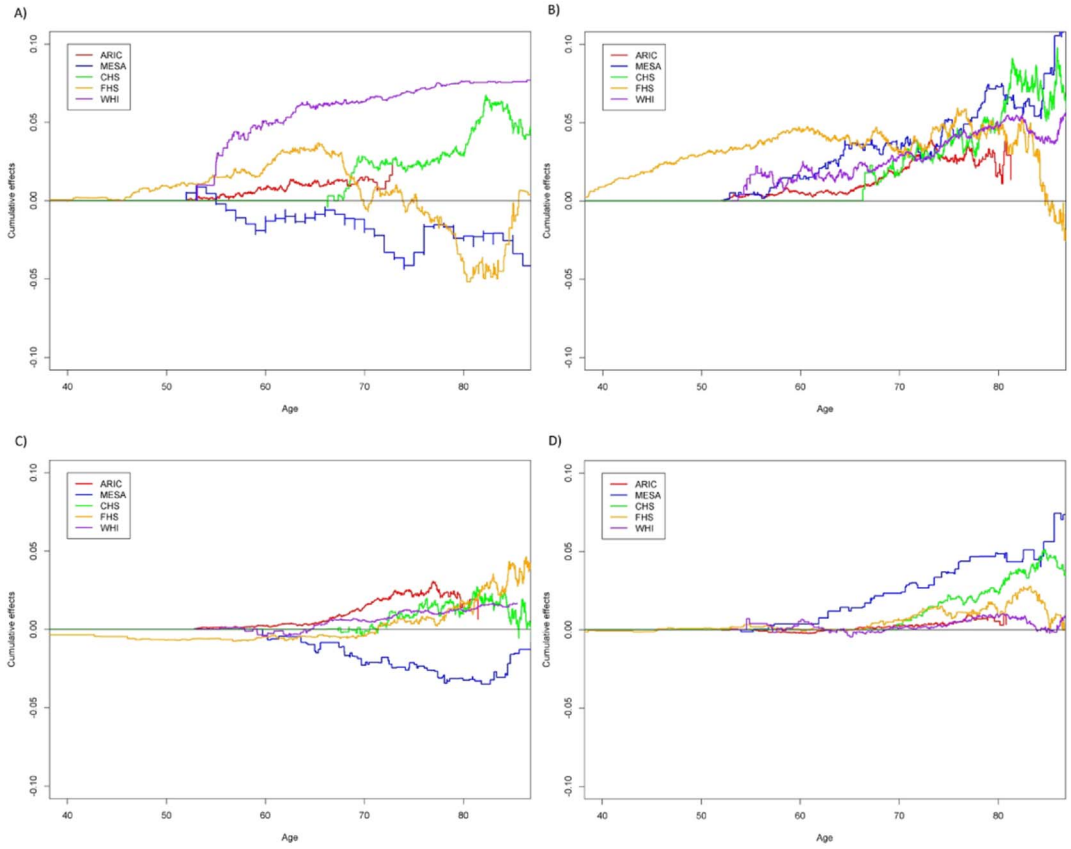


**Fig. 5.** Age-dependent cumulative causal effects of BMI on T2D, HF, CVD and stroke from age 40 to 85 in the five cohorts. Cumulative effect is the cumulative change of the absolute hazard with one unit change of the risk factor. A) BMI on T2D, B) BMI on CVD, C) BMI on HF, D) BMI on stroke.

before the measurement of the exposure, i.e., LDL-C in this case. The observation of an inverse association in our analysis of the FHS cohort seems not to support the reverse causality hypothesis because most measurements of LDL-C in FHS predate cancer diagnosis by three decades as shown in the density plot. Another potential explanation is the effect of treatments such as statin for controlling LDL-C levels. Nevertheless, it is still under debate whether treatment with statin has a beneficial or detrimental effect on the incidence of cancer (Jukema et al., 2012).

BP is one of the main risk factors for the development of AF and is involved in all of the underlying potential risk models for AF (Manolis et al., 2012). We found that the significant associations were observed in both cohort and the cumulative effects soared even at older age. This result seems to conflict with a latest large-scale association study using linked electronic health records, which shows that the significant effect of SBP attenuates with increasing age (Emdin et al., 2016).

Regarding causal effects on AD, our results confirm that the association between LDL-C and AD is no longer significant when excluding rs2075650 (in *TOMM40*) and rs10402271 (associated with *BCAM*). Both SNPs are close to *APOE*, which has been shown to have pleiotropic effects on lipids and AD (Østergaard et al., 2015; Proitsi et al., 2014). Unlike the previous findings about SBP (Østergaard et al., 2015), we did not observe the significant association between SBP and the hazards of AD despite the observation of the same direction of effect size.

The significant findings from the analyses using the polygenic scores are consistent with the results using the MR-Egger method. However, MR-Egger detected additional significant causal associations, including the causal effects of LDL-C on CHD, HF, CVD, stroke and MI. Although a recent study shows that MR-Egger may be biased and interpretation should be cautious (Burgess and Thompson, 2017), we notice that the major difference in these associations between MR-Egger and the method using polygenic scores results from different estimates in WHI, which only includes female individuals. As LDL-C has been widely reported to have causal influence on CHD (Ference et al., 2012; Holmes et al., 2015; Linsel-Nitschke et al., 2008) and MI (Voight et al., 2012), this indicates that direct use of the polygenic scores constructed from GWAS may not be powerful in an MR analysis for a specific sub-population.

Although much work has been done to minimize the bias and increase the statistical power, our MR study still has some limitations. First, the definitions of the diseases and criteria of diagnosis may vary across these five cohorts. This may explain some of the observed heterogeneity among these cohorts. Second, the constructed polygenic scores can only explain a small proportion of the variance of the intermediate risk factors, which may limits the statistical power to detect the causal effects. Third, the medication history during the follow-up can also affect the estimated effects of certain risk factors. Fourth, the numbers of cases for some diseases are small, so there might be no sufficient statistical power to detect the causal effect for these diseases.

In conclusion, we report significant causal associations between certain cardiovascular intermediate risk factors and the age-at-onset of age-related diseases. The estimated causal effects highlight BMI, which is significantly associated with increased risks of T2D, HF and CVD, implying its potential etiological role implicated in these diseases and the importance as the target for prevention procedures. SBP has a causal effect on AF, which manifests even after age ~80. In addition, we provide evidence to show that multiple previously reported associations are probably not driven by its biological causality, but rather result from the potential pleiotropy of certain genetic variants, either biological or spurious (pleiotropy due to design artefact or LD with two different causal variants (Solovieff et al., 2013)). The association of BMI with stroke may be partly attributed to the pleiotropic effects of multiple SNPs including rs2867125 and rs7359397. The identified inverse association between triglyceride levels and the onset of T2D may

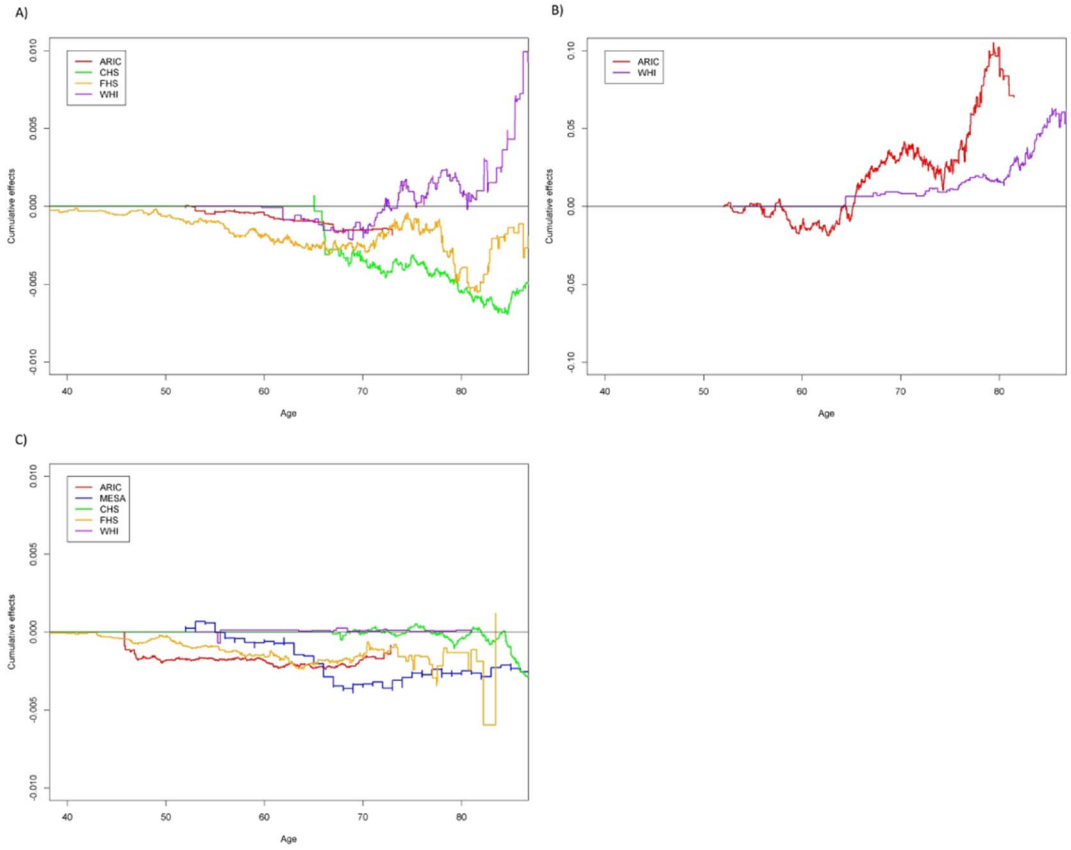


**Fig. 6.** Age-dependent cumulative causal effects of (A) LDL-C on cancer, (B) SBP on AF and (C) triglycerides on T2D from age 40 to 85. Cumulative effect is the cumulative change of the absolute hazard with one unit change of the risk factor. The AF variable is not available. Due to a numerical issue, we used a scaled SBP (0.1 ∗ SBP) when fitting the dynamic additive hazards model using *timereg*.

attribute to the pleiotropy of SNPs like rs1260326. These findings provide more insights into the biological mechanisms underlying the observed associations between these common risk factors and the risk of age-related diseases.

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