Molecular Medicine

Exploring the Causal Pathway From Telomere Length to Coronary Heart Disease
A Network Mendelian Randomization Study

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Rationale: Observational studies have found shorter leukocyte telomere length (TL) to be a risk factor for coronary heart disease (CHD), and recently the association was suggested to be causal. However, the relationship between TL and common metabolic risk factors for CHD is not well understood. Whether these risk factors could explain pathways from TL to CHD warrants further attention.

Objective: To examine whether metabolic risk factors for CHD mediate the causal pathway from short TL to increased risk of CHD using a network Mendelian randomization design.

Methods and Results: Summary statistics from several genome-wide association studies were used in a 2-sample Mendelian randomization study design. Network Mendelian randomization analysis—an approach using genetic variants as the instrumental variables for both the exposure and mediator to infer causality—was performed to examine the causal association between telomeres and CHD and metabolic risk factors. Summary statistics from the ENGAGE Telomere Consortium were used (n=37 684) as a TL genetic instrument, CARDIoGRAMplusC4D Consortium data were used (case=22 233 and control=64 762) for CHD, and other consortia data were used for metabolic traits (fasting insulin, triglyceride, total cholesterol, low-density lipoprotein cholesterol, fasting glucose, diabetes mellitus, glycohemoglobin, body mass index, waist circumference, and waist:hip ratio). One-unit increase of genetically determined TL was associated with −0.07 (95% confidence interval, −0.01 to −0.12; *P*=0.01) lower log-transformed fasting insulin (pmol/L) and 21% lower odds (95% confidence interval, 3–35; *P*=0.02) of CHD. Higher genetically determined log-transformed fasting insulin level was associated with higher CHD risk (odds ratio, 1.86; 95% confidence interval, 1.01–3.41; *P*=0.04).

Conclusions: Overall, our findings support a role of insulin as a mediator on the causal pathway from shorter telomeres to CHD pathogenesis. (*Circ Res*. 2017;121:214-219. DOI: 10.1161/CIRCRESAHA.116.310517.)

Key Words: body mass index ■ cardiovascular diseases ■ coronary disease ■ diabetes mellitus ■ insulin

Telomeres are repetitive nucleotide sequences $(TTAGGG)_n$ at the end of chromosomes that protect the chromosomes from aberration or fusion with each other. Telomeres are shortened during each cell division and are proposed as biomarkers of cellular senescence and biological aging.[1] Previous population-based observational studies found that shorter telomere length (TL) was associated with higher risk of coronary heart disease (CHD).[2,3] Moreover, a study using weighted telomere genetic risk scores (GRS) presented causal evidence that longer telomeres were associated with lower risk of CHD.[4] The finding was later confirmed in a recent study using a Mendelian randomization (MR) design.[5] However, the potential pathways involved in the association from telomeres to CHD have not yet been studied. Thus far, several traditional and newly emerging risk factors are associated with CHD risk,[6,7] some of which, such as glycemic traits, lipids, and obesity, are also closely related to telomere shortening.[8–11] Thus, these may act as potential mediators that lie in the pathway from telomere shortening to increased risk of CHD.

In this study, we aim to examine metabolic risk factors as possible mediators in the causal relationship between genetically determined TL and CHD using a network MR method[12] from summarized genome-wide association study (GWAS) data. The rationale for focusing on metabolic risk factors is based on the following: (1) these metabolic risk factors explain a large proportion

Novelty and Significance

What Is Known?

- Short telomere length is a causal risk factor for coronary heart disease (CHD).
- Several metabolic biomarkers are causally associated with CHD.

What New Information Does This Article Contribute?

- We investigated the pathway that links short telomeres to increased risk of CHD.
- The causal pathway from telomere attrition to CHD is partially mediated by fasting insulin level.

Short telomere length is associated with a higher risk of CHD. However, the mechanisms by which telomere attrition influences the risk of CHD is largely unknown, and from population-based studies, it is unclear whether metabolic biomarkers mediate this association. We used genetic variants associated with telomere length to test whether metabolic biomarkers are involved in the relationship between telomere length and CHD using network Mendelian randomization. Our results provide evidence to support the notion that fasting insulin level is a mediator that lies in the causal pathway from telomere shortening to CHD development. Interventions targeting fasting levels of insulin might partially counteract the detrimental effects of telomere shortening on CHD risk.

Nonstandard Abbreviations and Acronyms

CARDIoGRAM	coronary artery disease genome-wide replication and meta-analysis
CARDIoGRAMplusC4D	CARDIoGRAMplusC4D Metabochip and GWAS metabochip meta-analysis
CHD	coronary heart disease
CI	confidence interval
DIAGRAM	diabetes genetics replication and meta-analysis
ENGAGE	European Network for Genetic and Genomic Epidemiology
GIANT	genetic investigation of anthropometric traits
GLGC	global lipids genetics consortium
GRS	genetic risk score
GWAS	genome-wide association study
IV	instrument variable
LDL-C	low-density lipoprotein cholesterol
MAGIC	meta-analyses of glucose and insulin-related traits consortium
MR	Mendelian randomization
OR	odds ratio
SNP	single-nucleotide polymorphisms
TL	telomere length
WC	waist circumference
WHR	waist:hip ratio

of variance for CHD and (2) the GWAS summary statistics for these risk factors are publically available and can be used for MR analyses. The potential mediators include fasting insulin, fasting glucose, diabetes mellitus, glycohemoglobin, triglycerides, low-density lipoprotein cholesterol, total cholesterol, body mass index, waist circumference, and waist:hip ratio (WHR).

Methods

Summary of GWAS Data

Data included in this study were the GWAS summary statistics datasets from the ENGAGE Telomere Consortium[4] for TL; MAGIC Consortium (http://www.magicinvestigators.org/)[13,14] and DIAGRAM Consortium[15,16] for glycemic traits (http://diagram-consortium.org/index.html); GIANT Consortium (https://www.broadinstitute.org/collaboration/giant/index)[17] for body mass index, waist circumference, and WHR; global lipids genetics consortium (http://csg.sph.umich.edu//abecasis/public/lipids2013)[18] for lipids; ENGAGE 1000 Genome Consortium[19,20]

(http://diagram-consortium.org/2015_ENGAGE_1KG/) for fasting insulin, lipids, and WHR; and CARDIoGRAM Consortium and CARDIoGRAMplusC4D (http://www.cardiogramplusc4d.org/)[21,22] 1000 Genome Consortium for CHD. They were used in enrichment analyses and to obtain the effects of genetic variants on TL, metabolic risk factors, and CHD. A brief summary of these data is presented in Table 1 and Online Tables I through XI. There is no sample overlap between ENGAGE Telomere Consortium and CARDIoGRAM Consortium. The overlap for fasting insulin samples between the ENGAGE 1000 Genome

Table 1.   Summary Statistics Data Sources

Trait	Data Source	Total No. or Case/Control	Men, %
Telomere length	Codd; *Nat Genet*, 2013	37 684	42.4
CHD	Schunkert; *Nat Genet*, 2011	22 233/64 762	48.9
CHD	Nikpay; *Nat Genet*, 2015	60 801/123 504	…
Fasting insulin	Dupuis; *Nat Genet*, 2010	46 186	44.4
TG	Willer; *Nat Genet*, 2013	94 595	51.9
LDL	Willer; *Nat Genet*, 2013	94 595	51.9
TC	Willer; *Nat Genet*, 2013	94 595	51.9
Fasting glucose	Dupuis; *Nat Genet*, 2010	46 186	44.1
Diabetes mellitus	Morris; *Nat Genet*, 2012	12 171/56 862	41.4
HbA1c	Soranzo; *Diabetes*, 2010	46 368	48.0
BMI	Locke; *Nature*, 2015	322 154	47.4
WC	Shungin; *Nature*, 2015	224 459	44.4
WHR	Shungin; *Nature*, 2015	224 459	44.4
WHR	Horikoshi; *PLoS Genet*, 2015	54 572	47.2
Fasting insulin	Horikoshi; *PLoS Genet*, 2015	24 245	43.0
TG	Surakka; *Nat Genet*, 2015	62 166	44.6
LDL	Surakka; *Nat Genet*, 2015	62 166	44.6
TC	Surakka; *Nat Genet*, 2015	62 166	44.7

BMI indicates body mass index; CHD, coronary heart disease; HbA1c, glycohemoglobin; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; WC, waist circumference; and WHR, waist:hip ratio.

Consortium and the MAGIC Consortium is ≈10%. Approximately 40% of the lipid fractions samples in the ENGAGE 1000 Genome Consortium were in the global lipids genetics consortium. All GWAS summary statistic data were based on European ancestry populations except for CARDIoGRAMplusC4D 1000 Genome Consortium, which included Asian as well.

MR Analysis

MR can be used to assess the causal effect of an exposure on an outcome using genetic variants as instrument variables (IVs) in the association.[23] It has the advantage over observational studies in controlling for residual confounding and reverse causation. The rationale of an MR study is that if an exposure (eg, TL) is causally associated with an outcome (eg, CHD), then the genetic variants that determine the exposure should also be associated with the outcome. By examining the association between the genetic variants and the outcome, we can test whether an exposure is causally associated with an outcome. The MR analysis relies on 3 core assumptions: (1) the genetic variant is associated with the exposure, (2) the genetic variant is not associated with any confounders of the exposure and the outcome, and (3) the effect of the genetic variant on the outcome is completely through the exposure. For a 2-sample MR analysis using summary statistics, we constructed IVs using multiple genetic variants and used an inverse-variance weighted method (Online Data Supplement) to estimate the causal effect sizes as described previously.[24]

The framework of the network MR analysis is described in the Figure. It consists of 3 different MR tests that are all described below (I–III). First, the causal effect of genetically determined TL on CHD is estimated (I). Next, the causal effects of genetically determined TL on the metabolic risk factors—the potential mediators—are analyzed (II). Finally, the causal effects of the possible mediators on CHD are estimated (III). If causal associations are observed in all 3 steps, the conclusion can be drawn that the specific metabolic risk factor is a mediator.

For the first step (I), a GRS for TL was constructed as the IV from 7 single-nucleotide polymorphisms (SNPs; rs10936599, rs2736100, rs7675998, rs9420907, rs8105767, rs755017, and rs11125529) of genome-wide significance with TL in the GWAS from the ENGAGE Telomere Consortium (Online Table XII) to estimate the causal effect of genetically determined TL on CHD using the summary statistics from the CARDIoGRAMplusC4D Consortium.[21,22] The second step (II) used the same GRS as the IV for TL as described above and estimated the causal effect of genetically determined TL on fasting insulin, fasting glucose, diabetes mellitus, glycohemoglobin, triglycerides, low-density lipoprotein cholesterol, total cholesterol, body mass index, waist circumference, and WHR from the respective GWAS summary statistics. The last MR analyses (III) were performed for metabolic risk factors on CHD if TL was shown to have a causal effect on the risk factors in (II). Thus, we constructed a GRS for the metabolic risk factors that were tested further. The extent to which the association of TL with CHD was mediated by fasting insulin was tested in a post hoc analysis after fasting insulin was identified as the potential mediator (Online Data Supplement).

An MR analysis could be confounded by population stratification, which occurs when different subpopulations are included in the same analysis, for example, different ethnicities. Adjusting for principal components in the original GWAS analysis, which is a common practice, can minimize the effect. In this study, to minimize population stratification bias, which would also violate the MR assumptions, we only used data from participants of European ancestry. Moreover, the GWAS summary statistics used were also adjusted by principal components. Linkage disequilibrium may also bias the MR estimates when genetic variants correlated with the variants used in the analysis have effects on competing risk factors. Empirical testing of the association of known potential confounders with the variants can alleviate concerns about biased estimates. In practice, it is not necessary for the genetic variants used as instruments to be the causal variants. A variant in linkage disequilibrium with a causal variant is also a valid variant because it could still divide the population into subgroups that differ only in the exposure. This is illustrated in Online Figure I.

Sensitivity Analyses

Sensitivity analyses were performed to test the MR assumptions using a heterogeneity test proposed for this purpose.[25] The method assumes that all valid IVs should yield the same causal estimate. The associations of each SNP used for the GRS with the outcome should be proportional to their association with TL. Presence of any substantial heterogeneity would be suggestive of pleiotropic effects of the SNPs. Additional MR analyses were performed to examine whether the mediator (fasting insulin) could causally affect TL by exchanging TL and mediator (fasting insulin) and using the mediator (fasting insulin) GRS as the IV (Online Figure II). We also performed MR analyses using a SNP as IV (Online Figures III and IV). A *P* value <0.05 was considered as statistically significant.

Results

Causal Associations Between Genetically Determined TL and CHD

Table 2 lists the causal estimates between genetically determined TL and CHD from CARDIoGRAM and CARDIoGRAMplusC4D 1000 Genome Consortium. The
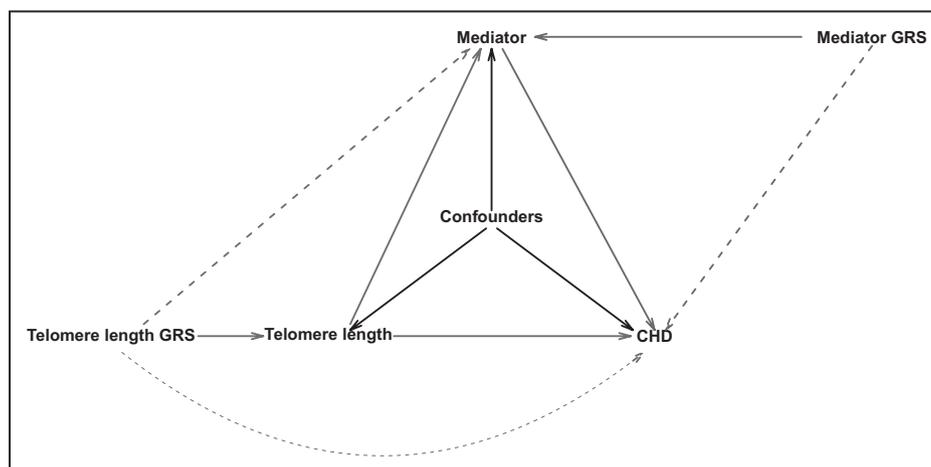

Figure. Network Mendelian randomization analysis framework. The solid lines depict the true potential causal diagram. The dashed lines represent the parameters that need to be estimated, which are equal to the multiplication of the respective effects represented by the solid lines. For instance, the dashed line from Telomere length genetic risk score (GRS) to Mediator means the total effect of Telomere length GRS on Mediator, which is equal to the effect of Telomere length GRS on Telomere length (solid line) multiplied by the effect of Telomere length on Mediator (solid line). CHD indicates coronary heart disease.

Table 2.   Causal Estimates for the Association Between Telomere Length and Coronary Heart Disease

Data Source	Causal Estimate		
	OR	95% CI	*P* Value
CARDIoGRAM CHD	0.79	0.65–0.97	0.016
CARDIoGRAMplus C4D 1000 Genome CHD	0.89	0.79–1.00	0.052
CARDIoGRAMplus C4D 1000 Genome MI	0.88	0.76–1.00	0.044

CARDIoGRAM indicates coronary artery disease genome-wide replication and meta-analysis; CHD, coronary heart disease; CI, confidence interval; MI, myocardial infarction; and OR, odds ratio.

causal estimate using 7 SNPs as IVs showed 1-unit (SD increase on T:S-ratio scale) longer genetically determined telomeres to be associated with lower risk of developing CHD (odds ratio [OR], 0.79; 95% confidence interval [CI], 0.65–0.97; *P*=0.016) in the CARDIoGRAM Consortium and (OR, 0.89; 95% CI, 0.79–1.00; *P*=0.052) in the CARDIoGRAMplusC4D 1000 Genome Consortium. Although the heterogeneity test of these 7 SNPs was not statistically significant (*P*=0.09), 1 SNP (rs7675998) was suspected to account for the heterogeneity because of its different effect on CHD compared with other SNPs. Additional MR analysis was, therefore, performed to calculate the estimate of TL on CHD by excluding rs7675998. This analysis yielded a similar effect size (OR, 0.71; 95% CI, 0.58–0.87; *P*=0.001) and less concern about the heterogeneity violation (*P*=0.59) for the remaining SNPs.

Causal Associations Between Genetically Determined TL and Metabolic Risk Factors

Table 3 describes causal estimates between genetically determined TL and metabolic biomarkers, including fasting insulin, triglycerides, total cholesterol, low-density lipoprotein cholesterol, fasting glucose, diabetes mellitus, glycohemoglobin, body mass index, waist circumference, and WHR. Because several large summary statistics from different consortia were available for the same biomarker, we used the earliest and largest summary data as the discovery data set and the most recent data—the ENGAGE 1000 Genome—as the replication data set. The MR analyses showed that TL was associated with fasting insulin, total cholesterol, triglycerides, and low-density lipoprotein cholesterol in the discovery phase. However, only the result for fasting insulin was replicated in the ENGAGE 1000 Genome Consortium.

Causal Association Between Genetically Determined Fasting Insulin and CHD

Based on the pathway analysis and MR investigation of the associations between TL and metabolic biomarkers, fasting insulin was suggested to be a potential mediator from shorter telomeres to increased risk of CHD. Thus, we evaluated further whether insulin was associated with CHD using MR analysis. First, we used 12 SNPs that were reported by the MAGIC Consortium to be associated with fasting insulin,[14] as the IVs. Increased log-transformed fasting insulin levels

Table 3.   Causal Estimates for the Association Between Telomere Length and Metabolic Risk Factors for Coronary Heart Disease

Trait	Causal Estimate		
	β	SE	*P* Value
Discovery			
Fasting insulin	−0.0679	0.027	0.011
TG	0.0796	0.027	0.003
LDL	0.0746	0.030	0.013
TC	0.0702	0.029	0.015
Fasting glucose	0.0236	0.026	0.361
Diabetes mellitus	−0.1205	0.122	0.322
HbA1c	0.0018	0.023	0.938
BMI	−0.0005	0.023	0.982
WC	0.026	0.027	0.324
WHR	0.050	0.026	0.056
Replication			
Fasting insulin	−0.0806	0.029	0.005
TG	0.0386	0.039	0.323
LDL	0.0736	0.039	0.062
TC	0.0453	0.039	0.240
WHR	0.014	0.041	0.720

BMI indicates body mass index; HbA1c, glycohemoglobin; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride; WC, waist circumference; and WHR, waist:hip ratio.

were associated with higher odds of CHD (OR, 2.64; 95% CI, 1.55–4.48; *P*=0.0004). Because of pleiotropic effects of rs1421085 (located within the FTO locus), which affects obesity, it was then excluded from the analysis. Analysis of the remaining SNPs yielded an effect size of 2.41 (95% CI, 1.37–4.26; *P*=0.003). Further excluding rs2972143 (suggested by the heterogeneity test) in *IRS-1,* revealed an OR of 1.86 (95% CI, 1.01–3.41; *P*=0.04). The heterogeneity test for testing the remaining SNPs in the score was not statistically significant (*P*=0.11; Online Figure V). Hence, fasting insulin might act as a mediator in the causal pathway from TL to CHD and accounts for 18.4% of the total effect of TL on CHD.

Discussion

In this study, we investigated the potential pathways mediating effects from TL to CHD, with specific emphasis on metabolic risk factors for CHD. We concluded that fasting insulin may be a mediator in the link between genetically determined TL and risk of CHD. This finding was obtained from a network MR design. One-unit increase of genetically determined TL was associated with −0.07 lower log-transformed fasting insulin and 21% lower odds of CHD, whereas higher log-transformed genetically determined fasting insulin was associated with higher CHD risk. To the best of our knowledge, this is the first study to address possible biological mechanisms in the causal pathway from TL to CHD, and the mediating effect of fasting insulin has never been shown before in population-based studies.

Although the underlying mechanisms from telomeres to CHD have been discussed briefly in previous epidemiologic studies,[2,3,5] a comprehensive description has not been reported. In this study, we used publicly available summary statistics from several genetic consortia and found pathway that might be involved in the association between TL and CHD. Short telomeres are associated with higher concentrations of fasting insulin, which could further increase CHD risk. Our findings were consistent with observational epidemiologic studies, which found associations between shorter telomeres and higher fasting insulin levels,[8,26] and increased fasting insulin as a well-known risk factor for CHD.[27]

As already alluded to above, several observational studies have examined the associations between TL and insulin, but also with insulin-like growth factor-1, and diabetes mellitus.[8,26,28–31] Most of these studies found TL to be inversely associated with insulin or diabetes mellitus, whereas others did not observe this relationship after multivariable adjustments. Conventional explanations for the association, such as shared common causes (oxidative stress and unhealthy lifestyle factors), were based on cross-sectional examinations of these biomarkers.[9] However, a prospective investigation with repeated measurements could offer a more reliable interpretation. Recently, a longitudinal study for an average of 12-year follow-up time using the Danish Twin Registry found that shorter telomeres affected insulin resistance but not vice versa.[26] Another study suggested that the mechanism for the association might be that short telomeres lead to premature β-cell dysfunction and death, followed by impaired insulin secretion and impaired glucose tolerance.[32] Conversely, elevated insulin secretion could increase oxidative stress and subsequent cardiovascular diseases.[33]

The main strength of our study is the large sample size accrued from the GWAS summary statistics, enabling us to examine the causal relationship between TL, metabolic risk factors, including fasting insulin, and CHD. The limitations mainly concern the assumptions for MR analyses and sample overlap between discovery and replication summary statistics from different consortia. First, the genetic variants used as IVs must have a strong association with the exposure variables. Here, the GRS we used for the respective trait in the analyses were significantly associated with TL (F statistics=404; $P=4.9×10^{-90}$) and insulin (F statistics=346; $P=2.5×10^{-77}$) and satisfied this assumption.[34] Second, the random assortment of alleles at birth should rule out confounding factors in the association between TL, fasting insulin, and CHD. We only included study participants of European ancestry in the MR analyses; hence, population stratification is less likely to be a problem. Third, the genetic variants selected as IVs should have a direct effect only on the exposure variable but not on other variables. The genetic variants of TL have been reported to be essential in the biological function of telomeres; thus, potential pleiotropy is less likely. Nevertheless, we found TL to have an effect on fasting insulin. However, this effect is not a violation of the third assumption about pleiotropy, which states that the effect of IV (eg, TL GRS) on the outcome (eg, CHD) was completely through the exposure (TL). The finding for fasting insulin lies in the pathway: TL GRS→TL →fasting insulin→CHD, in which the effect of TL GRS on CHD was still completely through TL. This is illustrated in the Figure (solid line from TL GRS to TL). Moreover, in an attempt to address additional potential pleiotropy issues, we performed sensitivity analyses examining the pleiotropic effects. Heterogeneity tests further showed there was no strong evidence of pleiotropy (Online Figure V).

To address sample overlap, we used the earliest and largest summary data as the discovery data set and the most recent summary data, from the ENGAGE 1000 Genome Consortium, as the replication data set. The only trait found significant in both discovery and replication was fasting insulin, whereas the results for lipid fractions did not pass replication. The significant finding for fasting insulin may be more robust and reliable because it has been replicated in a relatively new study sample. The fact that lipid fractions were not replicated may be because of power issues (62 166 participants in the ENGAGE 1000 Genome Consortium compared with 94 595 participants in the global lipids genetics consortium) because sample overlap should rather have driven the replication results toward the discovery. Nevertheless, there are advantages of performing 2-sample MR analyses using different samples, especially when there is no (eg, TL and CHD) or little overlap (eg, TL and fasting insulin). In particular, the winners' curse effect, in which the effect-size estimate of the genetic variant with the strongest association from a GWAS tends to be overestimated, is less likely to occur in 2-sample MR analysis.[35] Another obvious major strength using GWAS summary statistics is the increased statistical power, particularly when the outcome is a binary trait like CHD.[24] Nonetheless, future studies with similar or even larger sample size are warranted to validate this finding.

In this study, we found that the effect of genetically determined TL on CHD was partially mediated by fasting insulin. Our finding is novel and significant in terms of understanding how telomere shortening affects CHD. We provided evidence from population-based human studies to support this conclusion. The underlying aging process, measured here by TL, contributes to CHD; hence, if we can better understand the aging process, we can better monitor risk for aging diseases.

In summary, using a network MR approach, we provided evidence supporting a causal role of genetically determined short TL on increased risk of CHD, which may be partially mediated by increased fasting insulin levels. The mediating effect of fasting insulin was novel. Further large-scale studies or longitudinal studies with repeated measurements of TL, insulin, and CHD are warranted to validate these findings.

Disclosures

None.

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Circulation Research

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Exploring the Causal Pathway From Telomere Length to Coronary Heart Disease: A Network Mendelian Randomization Study

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Supplemental Material

Inverse-variance weighted estimator

The causal estimate from the inverse-variance weighted (IVW) method (β_{IVW}) is calculated by the following equation:

$$\beta_{IVW} = \frac{\sum_{i=1}^{N} \beta_{exp.i}\beta_{out.i}\sigma_{out.i}^{-2}}{\sum_{i=1}^{N} \beta_{exp.i}\sigma_{out.i}^{-2}}$$

The standard error is estimated by:

$$se(\beta_{IVW}) = \frac{1}{\sum_{i=1}^{N} \beta_{exp.i}\sigma_{out.i}^{-2}}$$

where N is the total number of genetic variants, $\beta_{exp.i}$ is the effect of i-th genetic variant on the exposure, $\beta_{out.i}$ is the i-th genetic variant on the outcome, and $\sigma_{out.i}$ is the standard error of $\beta_{out.i}$.

Mediation analysis

The extent to which the association of TL with CHD was mediated by fasting insulin was tested in a post-hoc analysis after fasting insulin was identified as the potential mediator. The total effect (odds ratio: OR) of 1 SD increase of TL on CHD was 0.79 [log(OR)= -0.236]. The effect of 1 SD increase of TL on fasting insulin was -0.07, and 1 unit increase in fasting insulin was associated with CHD [log(OR)=log(1.86)=0.62]. Thus, the mediated effect of fasting insulin was -0.07×0.62=-0.043. The mediated proportion was -0.043/-0.236 = 18.4%.

Online Figure I. Illustration of the MR analysis when a genetic variant is in linkage disequilibrium with a causal variant. The dashed line indicates that the two variants (a causal variant and a measured variant) that are in LD are correlated. The solid headed lines stand for the causal association.
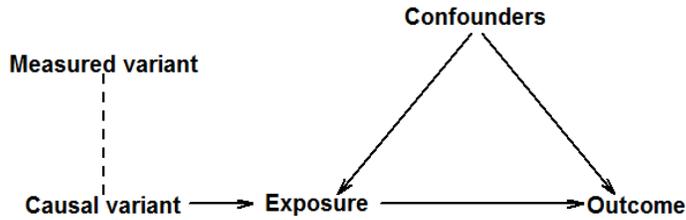

Online Figure II. Examining the causal effects of fasting insulin on telomere length using Mendelian randomization design.

By exchanging TL and fasting insulin in main text Figure 1, we aimed to examine if fasting insulin could causally affect CHD via TL. The causal effect of fasting insulin on CHD has been presented in the main text, which shows fasting insulin causes CHD. Now the next step was to assess if fasting insulin was causally associated with TL (the solid red line from fasting insulin to telomere). By using 12 SNPs as the instrumental variable for fasting insulin, we found no evidence to support a causal association (β=0.11, 95% CI: -0.16, 0.38, P=0.42). Additionally excluding the SNP in FTO locus, similar results were obtained (β=0.10, 95% CI: -0.19, 0.40, P=0.48). Thus, we conclude TL does not mediate the effect from fasting insulin to CHD.
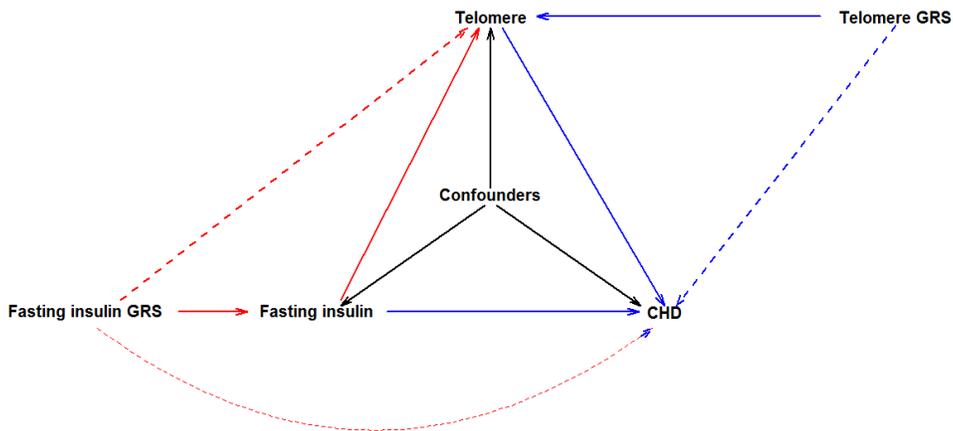
Online Figure III. MR estimates of telomere length on fasting insulin (left panel) and coronary heart disease (right panel) by using both a single SNP and genetic risk score(GRS) as instrumental variables.
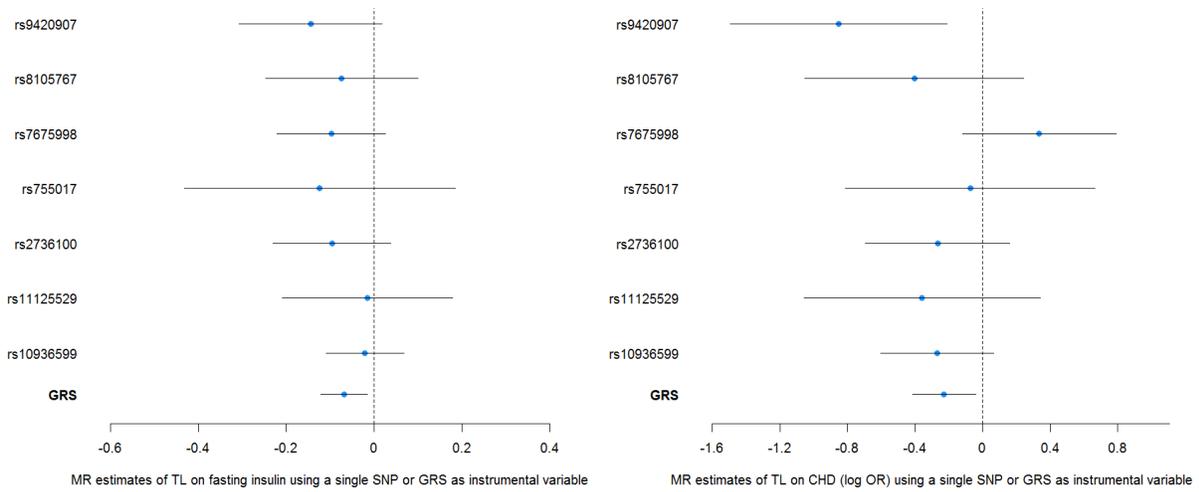
Online Figure IV. MR estimates of fasting insulin on coronary heart disease by using both a single SNP and genetic risk score(GRS) as instrumental variables.

MR estimates of fasting insulin on CHD using a single SNP or GRS as instrumental variable
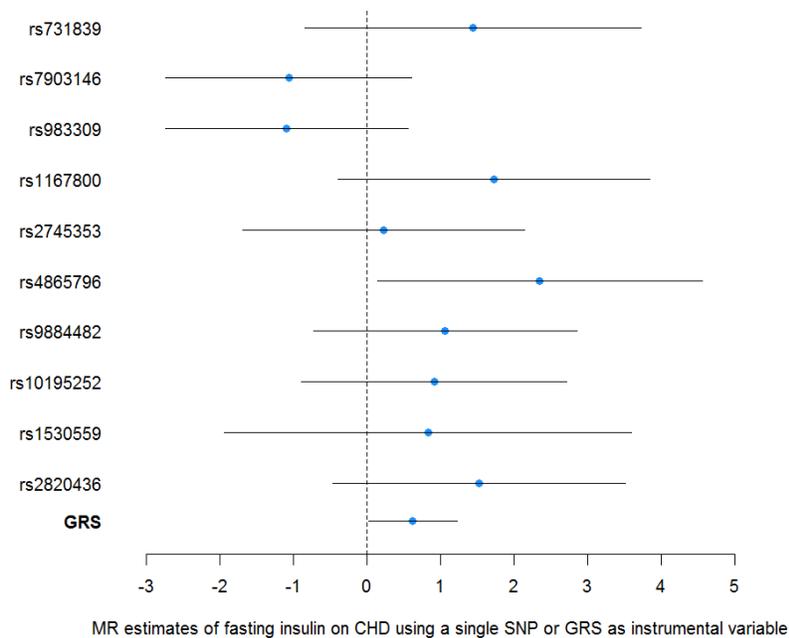
Online Figure V. Scatter plots showing the per-allele association with insulin plotted against the per-allele association with telomere length (top left panel), coronary heart disease plotted against the per-allele association with telomere length (top right panel), coronary heart disease plotted against the per-allele association with insulin (bottom left panel), and coronary heart disease plotted against the per-allele association with insulin additionally excluding two outliers (bottom right panel) with vertical lines showing 95% confidence interval for each SNP.