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

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Running title: Pathways from Telomeres to Heart Disease

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ABSTRACT

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

**Objective:** To examine if metabolic risk factors for CHD mediate the causal pathway from short telomere length to increased risk of CHD using a network Mendelian randomization (MR) design.

**Methods and Results:** Summary statistics from several genome-wide association studies were used in a two-sample MR study design. Network MR 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 as well as metabolic risk factors. Summary statistics from the ENGAGE Telomere Consortium were used (n=37,684) as a telomere length (TL) genetic instrument, CARDioGRAMplusC4D Consortium data were used (case=22,233, 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, HbA1c, body mass index, waist circumference, and waist to hip ratio). One unit increase of genetically determined TL was associated with -0.07 (95% confidence interval: -0.01, -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.

**Keywords:** Telomere length, coronary heart disease, insulin, Mendelian randomization, cardiovascular disease, pathways analysis

**Nonstandard Abbreviations and Acronyms:**

- CHD: coronary heart disease
- CARDioGRAM: Coronary ARtery DIsease Genome-wide Replication And Meta-analysis
- CARDioGRAMplusC4D: CARDioGRAMplusC4D Metabochip and GWAS Metabochip meta-analysis
- 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 studies
- 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 to hip ratio
INTRODUCTION

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. Previous population-based observational studies found that shorter telomere length (TL) was associated with higher risk of coronary heart disease (CHD). Moreover, a study using weighted telomere genetic risk scores (GRS) presented causal evidence that longer telomeres were associated with lower risk of CHD. The finding was later confirmed in a recent study using a Mendelian randomization (MR) design. 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, some of which, such as glycemic traits, lipids, and obesity, are also closely related to telomere shortening. 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 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 of variance for CHD; 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, HbA1c, triglycerides (TG), low density lipoprotein cholesterol (LDL), total cholesterol (TC), body mass index (BMI), waist circumference (WC), and waist to hip ratio (WHR).

METHODS

Summary GWAS data.

Data included in this study were the GWAS summary statistics datasets from the ENGAGE Telomere Consortium for TL, MAGIC Consortium for glycemic traits, GIANT Consortium for BMI, WC, and WHR, Global Lipids Genetics Consortium for lipids, ENGAGE 1000 Genome Consortium for fasting insulin, lipids, and WHR, and CARDIoGRAM Consortium and CARDIoGRAMplusC4D 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-XI. There is no sample overlap between ENGAGE Telomere Consortium and CARDIoGRAM Consortium. The overlap for fasting insulin samples between the ENGAGE 1000 Genome Consortium and the MAGIC Consortium is around 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.

Mendelian randomization analysis.

Mendelian randomization can be used to assess the causal effect of an exposure on an outcome using genetic variants as instrument variables (IVs) in the association. 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 (e.g. TL) is causally associated with an outcome (e.g. 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 if an exposure is causally associated with an outcome. The MR analysis relies on three 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; 3) the effect of
the genetic variant on the outcome is completely through the exposure. For a two-sample MR analysis using summary statistics, we constructed IVs using multiple genetic variants and used an inverse-variance weighted method (Supplemental Material) to estimate the causal effect sizes as previously described.

The framework of the network MR analysis is described in Figure 1. It consists of three 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 three 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 seven 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. 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, HbA1c, TG, LDL, TC, BMI, WC, and WHR from the respective GWAS summary statistics. The last MR analyses (III) were carried out 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 factor 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 (Supplemental Material).

An MR analysis could be confounded by population stratification, which occurs when different sub-populations are included in the same analysis, e.g., different ethnicities. Adjusting for principal components in the original GWAS analysis, which is 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 (LD) 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 LD 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 carried out to test the MR assumptions using a heterogeneity test proposed for this purpose. 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 evidence of pleiotropic effects of the SNPs. Additional MR analyses were performed to examine if 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 single SNP as IV (Online Figure III and IV). A p-value less than 0.05 was considered as statistically significant.
RESULTS

Causal associations between genetically determined telomere length and coronary heart disease.

Table 2 lists the causal estimates between genetically determined TL and CHD from CARDioGRAM and CARDioGRAMplusC4D 1000 Genome Consortium. The causal estimate using seven SNPs as IV’s showed one unit (standard deviation 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-value=0.016] in the CARDioGRAM Consortium and [OR=0.89, 95% CI: 0.79-1.00, p-value=0.052] in the CARDioGRAMplusC4D 1000 Genome Consortium. Although the heterogeneity test of these seven SNPs was not statistically significant (p-value=0.09), one SNP (rs7675998) was suspected to account for the heterogeneity due to 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-value=0.001) and less concern about the heterogeneity violation (p-value=0.59) for the remaining SNPs.

Causal associations between genetically determined telomere length and metabolic risk factors.

Table 3 describes causal estimates between genetically determined TL and metabolic biomarkers including fasting insulin, TG, TC, LDL, fasting glucose, diabetes; HbA1c, BMI, WC, 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 dataset and the most recent data, the ENGAGE 1000 Genome, as the replication dataset. The MR analyses showed that TL was associated with fasting insulin, TC, TG, and LDL 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 coronary heart disease.

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. Firstly, we used 12 SNPs that were reported by the MAGIC Consortium to be associated with fasting insulin14, as the IVs. Increased log-transformed fasting insulin levels were associated with higher odds of CHD (OR 2.64, 95% CI: 1.55-4.48, p-value=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-value=0.003). Further excluding rs2972143 (suggested by the heterogeneity test) in IRS1, revealed an OR of 1.86 (95% CI: 1.01-3.41, p-value=0.04). The heterogeneity test for testing the remaining SNPs in the score was not statistically significant (p-value = 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, while higher log-transformed genetically

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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 epidemiological studies, a comprehensive description has not been reported. In this study, we utilized 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 epidemiological studies, which found associations between shorter telomeres and higher fasting insulin levels, and increased fasting insulin as a well-known risk factor for CHD.

As already alluded to above, a number of observational studies have examined the associations between TL and insulin, but also with insulin-like growth factor-1 (IGF-1), and diabetes. Most of these studies found TL to be inversely associated with insulin or diabetes, while 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. However, a prospective investigation with repeated measurements could offer a more reliable interpretation. Recently, a longitudinal study over an average of 12 years follow-up time using the Danish Twin Registry found that shorter telomeres affected insulin resistance but not vice versa. 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. Conversely, elevated insulin secretion could increase oxidative stress and subsequent cardiovascular diseases.

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 employed 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 ($F$-statistic=404, $p$-value=$4.9 \times 10^{-90}$) and insulin ($F$-statistic=346, $p$-value=$2.5 \times 10^{-77}$) and satisfied this assumption. 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 the effect of IV (e.g. TL GRS) on the outcome (e.g. CHD) was completely through the exposure (TL). The finding for fasting insulin lies in the pathway: TL $\rightarrow$ fasting insulin $\rightarrow$ CHD, in which the effect of TL GRS on CHD was still completely through TL. This is illustrated in Figure 2 (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 dataset and the most recent summary data, from the ENGAGE 1000 Genome Consortium, as the replication dataset. 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 due to 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 towards the discovery. Nevertheless, there are advantages of performing two-sample MR analyses using different samples, especially when there is no (e.g. TL and CHD) or little overlap (e.g. 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 two-sample MR analysis35. Another obvious major strength using GWAS summary statistics is the increased statistical power, particularly when the outcome is a binary trait like CHD24. Nonetheless, future studies with similar or even larger samples size are warranted to validate this finding.

In this study, we found 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.

SOURCES OF FUNDING
This study was funded by a Karolinska Institutet definsiering (KID) grant for doctoral student (YZ), the Loo & Hans Osterman Foundation, the Foundation for Geriatric Diseases, the Magnus Bergwall Foundation, the Gun and Bertil Stohnes Foundation, the Foundation for Gamla Tjänarinnor, the Swedish Council for Working Life and Social Research (2013-2292), the Swedish Research Council (521-2013-8689; 2015-03255), and the KI Foundation.

DISCLOSURES
None.

ONLINE RESOURCES
CARDIoGRAMplusC4D http://www.cardiogramplusc4d.org/;
DIAGRAM http://diagram-consortium.org/index.html;
MAGIC http://www.magicinvestigators.org/;
GIANT https://www.broadinstitute.org/collaboration/giant/index;
ENGAGE 1KG http://diagram-consortium.org/2015_ENGAGE_1KG/
REFERENCES


TABLE LEGENDS

Table 1. Summary statistics data sources

Table 2. Causal estimates for the association between telomere length and coronary heart disease

Table 3. Causal estimates for the association between telomere length and metabolic risk factors for coronary heart disease

FIGURE LEGENDS

Figure 1. 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 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 red line) multiplied by the effect of ‘Telomere length’ on ‘Mediator’ (solid red line). GRS: genetic risk score; CHD: coronary heart disease.
NOVELTY AND SIGNIFICANCE

What Is Known?

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

What New Information Does This Article Contribute?

- We investigated the pathway that link 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.
Table 1. Summary statistics data sources

<table>
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<tr>
<th>Trait</th>
<th>Data source</th>
<th>Total number or Case/Control</th>
<th>Men (%)</th>
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<td>Telomere Length</td>
<td>Codd, Nat Genet 2013</td>
<td>37 684</td>
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<td>CHD</td>
<td>Schunkert, Nat Genet 2011</td>
<td>22 233/64 762</td>
<td>48.9</td>
</tr>
<tr>
<td>CHD</td>
<td>Nikpay. Nat Genet 2015</td>
<td>60 801/123 504</td>
<td>-</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>Dupuis, Nat Genet 2010</td>
<td>46 186</td>
<td>44.4</td>
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<td>TG</td>
<td>Willer, Nat Genet 2013</td>
<td>94 595</td>
<td>51.9</td>
</tr>
<tr>
<td>LDL</td>
<td>Willer, Nat Genet 2013</td>
<td>94 595</td>
<td>51.9</td>
</tr>
<tr>
<td>TC</td>
<td>Willer, Nat Genet 2013</td>
<td>94 595</td>
<td>51.9</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>Dupuis, Nat Genet 2010</td>
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<td>Diabetes</td>
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<td>HbA1c</td>
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<td>BMI</td>
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<td>WC</td>
<td>Shungin, Nature 2015</td>
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<td>WHR</td>
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<td>WHR</td>
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<td>TG</td>
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<td>LDL</td>
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<td>TC</td>
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<td>62 166</td>
<td>44.7</td>
</tr>
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</table>

CHD, coronary heart disease; TG, triglyceride; TC, total cholesterol; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio.
Table 2. Causal estimates for the association between telomere length and coronary heart disease

<table>
<thead>
<tr>
<th>Data source</th>
<th>Causal estimate</th>
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<tr>
<td>CARDioGRAM CHD</td>
<td>0.79 0.65, 0.97</td>
</tr>
<tr>
<td>CARDioGRAMplus C4D 1000 Genome CHD</td>
<td>0.89 0.79, 1.00</td>
</tr>
<tr>
<td>CARDioGRAMplus C4D 1000 Genome MI</td>
<td>0.88 0.76, 1.00</td>
</tr>
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</table>

CHD, coronary heart disease; MI, myocardial infarction; OR, odds ratio
### Table 3. Causal estimates for the association between telomere length and metabolic risk factors for CHD

<table>
<thead>
<tr>
<th>Trait</th>
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<th>Replication</th>
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</thead>
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<tr>
<td></td>
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<tr>
<td>Fasting insulin</td>
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<tr>
<td>TG</td>
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<td>LDL</td>
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<td>TC</td>
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<tr>
<td>Fasting glucose</td>
<td>0.0236</td>
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<td>Diabetes</td>
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<td>HbA1c</td>
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<td>0.023</td>
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<td>BMI</td>
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<td>WC</td>
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<td>WHR</td>
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<td>TC</td>
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<td>WHR</td>
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TL: telomere length, TG: triglyceride, LDL: low density lipoprotein, TC: total cholesterol, HbA1c: Glycated hemoglobin, BMI: body mass index, WC, waist circumference; WHR, waist to hip ratio; GIANT: Genetic Investigation of ANthropometric Traits; ENGAGE: European Network for Genetic and Genomic Epidemiology.
FIGURE 1

Diagram showing the relationship between telomere length, GRS, Mediator, Mediator GRS, Confounders, and CHD.
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Data Supplement (unedited) at:
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Supplemental Material

**Online Table I.** Basic characteristics of the ENGAGE Telomere Consortium

**Online Table II.** Basic characteristics of the CARDIoGRAM Consortium

**Online Table III.** Basic characteristics of the CARDIoGRAMplusC4D 1000 Genome Consortium

**Online Table IV.** Basic characteristics of the MAGIC Consortium

**Online Table V.** Basic characteristics of the DIAGRAM fasting glucose Consortium

**Online Table VI.** Basic characteristics of the GIANT BMI Consortium

**Online Table VII.** Basic characteristics of the GIANT waist circumference Consortium

**Online Table VIII.** Basic characteristics of the ENGAGE fasting insulin Consortium

**Online Table IX.** Basic characteristics of the DIAGRAM diabetes Consortium

**Online Table X.** Basic characteristics of the ENGAGE lipid Consortium

**Online Table XI.** Basic characteristics of the Global Lipid Genetics Consortium

**Online Table XII.** SNPs used to construct the genetic risk score. In the analysis of TL with metabolic risk factors, we only found consistent association of TL with fasting insulin. Thus, we constructed another GRS for the metabolic risk factors that were further tested for fasting insulin using 12 SNPs reported in the MAGIC consortium.

**Online Figure I.** Illustration of the MR analysis when a genetic variant is in linkage disequilibrium with a causal variant.

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

**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.

**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 insulin (bottom left panel), and coronary heart disease plotted against the per-allele association insulin (bottom right panel with additionally excluding two outliers) with vertical lines showing 95% confidence interval for each SNP.
**Inverse-variance weighted estimator**

The causal estimate from the inverse-variance weighted (IVW) method ($\beta_{IVW}$) 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 ($\beta=0.11$, 95% CI: -0.16, 0.38, $P=0.42$). Additionally excluding the SNP in FTO locus, similar results were obtained ($\beta=0.10$, 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.
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.