



Kunutsor, S. K. (2015). Serum total bilirubin levels and coronary heart disease - Causal association or epiphenomenon? *Experimental Gerontology*, 72, 63-6. DOI: [10.1016/j.exger.2015.09.014](https://doi.org/10.1016/j.exger.2015.09.014)

Peer reviewed version

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Serum total bilirubin levels and coronary heart disease – causal association or epiphenomenon?

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Abstract

Observational epidemiological evidence supports a linear inverse and independent association between serum total bilirubin levels and coronary heart disease (CHD) risk, but whether this association is causal remains to be ascertained. A Mendelian randomization approach was employed to test whether serum total bilirubin is causally linked to CHD. The genetic variant rs6742078 - well known to specifically modify levels of serum total bilirubin and accounting for up to 20% of the variance in circulating serum total bilirubin levels - was used as an instrumental variable. In pooled analysis of estimates reported from published genome-wide association studies, every copy of the T allele of rs6742078 was associated with 0.42 standard deviation (SD) higher levels of serum total bilirubin (95% confidence interval, 0.40 to 0.43). Based on combined data from the Coronary Artery Disease Genome wide Replication and Meta-analyses and the Coronary Artery Disease (C4D) Genetics Consortium involving a total of 36 763 CHD cases and 76 997 controls, the odds ratio for CHD per copy of the T allele was 1.01 (95% confidence interval, 0.99 to 1.04). The odds ratio of CHD for a 1 SD genetically elevated serum total bilirubin level was 1.03 (95% confidence interval, 0.98 to 1.09). The current findings casts doubt on a strong causal association of serum total bilirubin levels with CHD. The inverse associations demonstrated in observational studies may be driven by biases such as unmeasured confounding and/or reverse causation. However, further research in large-scale consortia is needed.

Keywords: Bilirubin; coronary heart disease; Mendelian randomization

Abbreviations

BMI body mass index

CARDIoGRAM Coronary Artery Disease Genome wide Replication and Meta-analyses

CHD coronary heart disease

CI confidence interval

CVD cardiovascular disease

GIANT Genetic Investigation of ANthropometric Traits

GLGC Global Lipids Genetics Consortium

GWAS genome-wide association studies

HDL high-density lipoprotein

HOMA-B homeostatic model assessment of beta-cell function

HOMA-IR homeostatic model assessment of insulin resistance

ICBP International Consortium for Blood Pressure

MAGIC Meta-Analyses of Glucose and Insulin-related traits Consortium

MR Mendelian randomization

OR odds ratio

SNP single-nucleotide polymorphism

SD standard deviation

SBP systolic blood pressure

TG triglycerides

WHR waist-to-hip ratio

Introduction

There is an ongoing debate on the potential value of serum total bilirubin levels in cardiovascular disease (CVD) risk prevention (i.e., either as a causal therapeutic target or as a marker of risk prediction (1)). Recently, in a comprehensive assessment of the association of baseline serum total bilirubin levels with risk of future first-ever CVD events in the general population using a large population-based cohort study, a linear inverse and independent association has been demonstrated.(2) In the same study, a multivariate adjusted relative risk [95% confidence interval (CI)] for CHD of 0.95 (0.92 to 0.99) per 1 standard deviation (SD) increase in total bilirubin levels in pooled analysis of 8 population-based prospective studies was reported. The evidence is suggestive of causality, but it is not possible to make causal inferences using observational epidemiological studies, as such data are beset by residual confounding and reverse causation.(3, 4) In the absence of randomized controlled trials that offer the highest clinical evidence for assessing causality, integrative studies of genetic variants [single-nucleotide polymorphisms (SNPs) specifically related to serum bilirubin levels may provide another route to help judge whether bilirubin could be directly causal in CHD (i.e., “Mendelian randomization [MR] analysis”(5)).

Serum bilirubin is under strong genetic regulation and shows a substantial variation among individuals. There are indications that a single gene locus [the uridine diphosphate glucuronyltransferase 1A1 (*UGT1A1*) on chromosome 2] accounts for a substantial proportion of the variation in serum bilirubin levels and is its major determinant.(6) A functional TATA box thymine adenine (TA) repeat variant in the promoter region of the *UGT1A1* gene - *UGT1A1**28 TATA box polymorphism - is known to significantly reduce *UGT1A1* production and activity and is associated with unconjugated hyperbilirubinemia.(7, 8) There are suggestions that this TA repeat variant might be the key polymorphism within

the *UGT1A1* gene controlling serum bilirubin levels.(9) It has been hypothesized that the *UGT1A1**28 allele may be a protective factor against CHD, which may provide support for a causal relationship between bilirubin and CHD risk. However, association studies investigating the *UGT1A1* locus have provided conflicting but mostly null evidence for a potential protective effect of the *UGT1A1**28 allele on CHD.(7, 8) The rs6742078 variant in the *UGT1A1* gene [which has been shown to be in strong linkage disequilibrium with the *UGT1A1**28 allele(10)] is well known to robustly and specifically modify levels of circulating serum levels of total bilirubin (explaining up to 20% of the variation in total bilirubin levels(11)) and has been used as an instrument for examining the causal relevance of serum total bilirubin to disease outcomes.(11, 12) Stender and colleagues(12) have recently employed a MR approach using this variant as an instrumental variable and suggested a non-causal association between total bilirubin and CHD risk. The authors called for further work to extend these findings. Using large-scale genetic data with increased power, this study aimed to assess the association of the *UGT1A1* variant rs6742078 with CHD risk by utilising a MR approach.

Methods and materials

The rs6742078 was a suitable instrumental variable for the present analyses, given its robust specificity for serum total bilirubin levels and its use in previous MR studies.(11, 12) Estimates of the association of the genetic variant rs6742078 with CHD were extracted from publicly available data from two genetic consortia, comprising the Coronary Artery Disease Genome wide Replication and Meta-analyses (CARDIoGRAM),(13) and the Coronary Artery Disease (C4D) Genetics consortium.(14) Estimates of power to detect associations with CHD employed Purcell's online power calculation for genetic studies.(15) Available genome-wide association studies (GWASs) reporting on the associations of the rs6742078

variant (or proxies, $r^2 = 1.0$ with the index SNP based on the CEU Hap Map population) with serum total bilirubin levels at genome-wide significant levels ($P < 5 \times 10^{-8}$) were included in the present analyses. Studies were identified by searching the original publications of GWASs for serum total bilirubin levels that have been indexed by the National Human Genome Research Institute (NHGRI) GWAS catalogue.(16)

The rs6742078 variant has been robustly demonstrated not to be associated with several cardiovascular risk markers that might confound the relationship between serum total bilirubin and CHD risk.(17, 18) To further assess the scope for pleiotropic effects, associations of the rs6742078 variant with several cardiometabolic traits were explored using data from published GWASs available from <http://csg.sph.umich.edu/locuszoom/>.(19) A MR approach was employed which was based on the use of summary estimates for both rs6742078-serum total bilirubin and rs6742078-CHD associations. The MR estimate was derived using the Wald-type estimator,(20) given as:

$$\log \text{OR}_{\text{CHD/serum total bilirubin}} = \log \text{OR}_{\text{CHD/allele}} / \beta_{\text{serum total bilirubin/allele}}$$

Where $\log \text{OR}_{\text{CHD/serum total bilirubin}}$ is the (log) increase of CHD risk per standard deviation (SD) increase in serum total bilirubin (MR estimate), $\log \text{OR}_{\text{CHD/allele}}$ is the (log) increase in CHD risk per allele (rs6742078-CHD association), and $\beta_{\text{serum total bilirubin/allele}}$ is the number of SDs above the mean serum total bilirubin level per allele (rs6742078-serum total bilirubin association). The standard error of the MR estimate was estimated using the Delta method.(21, 22)

Results

As expected, there was no evidence of associations of the rs6742078 variant with any of the cardiovascular risk markers assessed (**Figure 1**). An estimate of the effect of the variant on

serum total bilirubin levels was based on pooled analysis of estimates reported from previous GWASs (**Table 1**). The pooled effect on serum total bilirubin levels, expressed as per SD higher change, was 0.42 (95% CI, 0.40-0.43) for each copy of the T allele, with estimates ranging from 0.29 to 0.57 SD across studies. The estimate of the effect on CHD risk was based on combined data from CARDIoGRAM and C4D consortia comprising of 36,763 CHD cases and 76,997 controls. The pooled OR for CHD per copy of the T allele was 1.01 (95% CI, 0.99-1.04). The statistical power of the genotype-CHD association to reliably detect the anticipated OR per minor allele was 94% (minor allele frequency for rs6742078 was 33%). The causal OR for CHD was estimated as 1.03 (95% CI, 0.98-1.09) per 1 SD increase in serum total bilirubin level, which is in contrast to the pooled estimate of 0.95 (95% CI, 0.92-0.99) from observational evidence (2) (**Figure 2**).

Discussion

Utilizing large-scale genetic data and a MR approach, the MR estimate showed no strong evidence of a causal effect of serum total bilirubin on risk of CHD, which is consistent with results of the recent MR study,(12) and in contrast to epidemiological evidence from observational studies.(2) The current findings are important and less prone to bias than results from traditional observational epidemiology, because causal investigations with the use of the *UGT1A1* variant are likely to be free from confounding, not subject to reverse-causation, and genetically-elevated serum levels of total bilirubin are indicative of life-long levels.

The analysis presented has strengths and limitations which merit consideration. The rs6742078 variant was found to be particularly informative because it is exclusively associated with substantial increases in total bilirubin. Additionally, a comprehensive assessment of the independence of this variant was conducted using published data from

several large-scale GWASs. Though this analysis was more adequately powered than the previous study, both analyses employed a single instrumental variable MR approach which may have limited the findings, given the lack of specificity often observed with single SNPs.(23) Mendelian randomisation analyses using multiple SNPs that are each inherited independently and affecting serum total bilirubin levels additively (allele scores) may be needed to rule out any causal relevance of serum total bilirubin levels to CHD risk. Furthermore, in the current analyses, a MR approach was employed using summarized published and publicly available data which precluded the ability to fully assess instrumental variable assumptions; adequately address population stratification; test for the attenuation of genetic associations with the outcome on adjustment for the exposure of interest; and assess parametric assumptions required by instrumental variable methods for effect estimation.(24) However, it has been reported that causal estimates from summarized data are almost as precise as those obtained from individual-level data.(24) The results should still be interpreted in context of these limitations. MR investigations using individual-level data may provide another efficient method to help establish or rule out causality.

In conclusion, the current findings do not provide strong evidence for a causal association between serum total bilirubin levels and CHD. The inverse associations demonstrated in observational studies may be driven by biases such as unmeasured confounding and/or reverse causation. However, given the limitations of the present study and that serum total bilirubin remains a promising though unproven strategy in the prevention of CHD, further evaluation may be warranted.

Acknowledgements

Data on coronary artery disease / myocardial infarction have been contributed by

CARDIoGRAMplusC4D investigators and have been downloaded from

www.CARDIOGRAMPLUSC4D.ORG

Conflicts of Interest: None

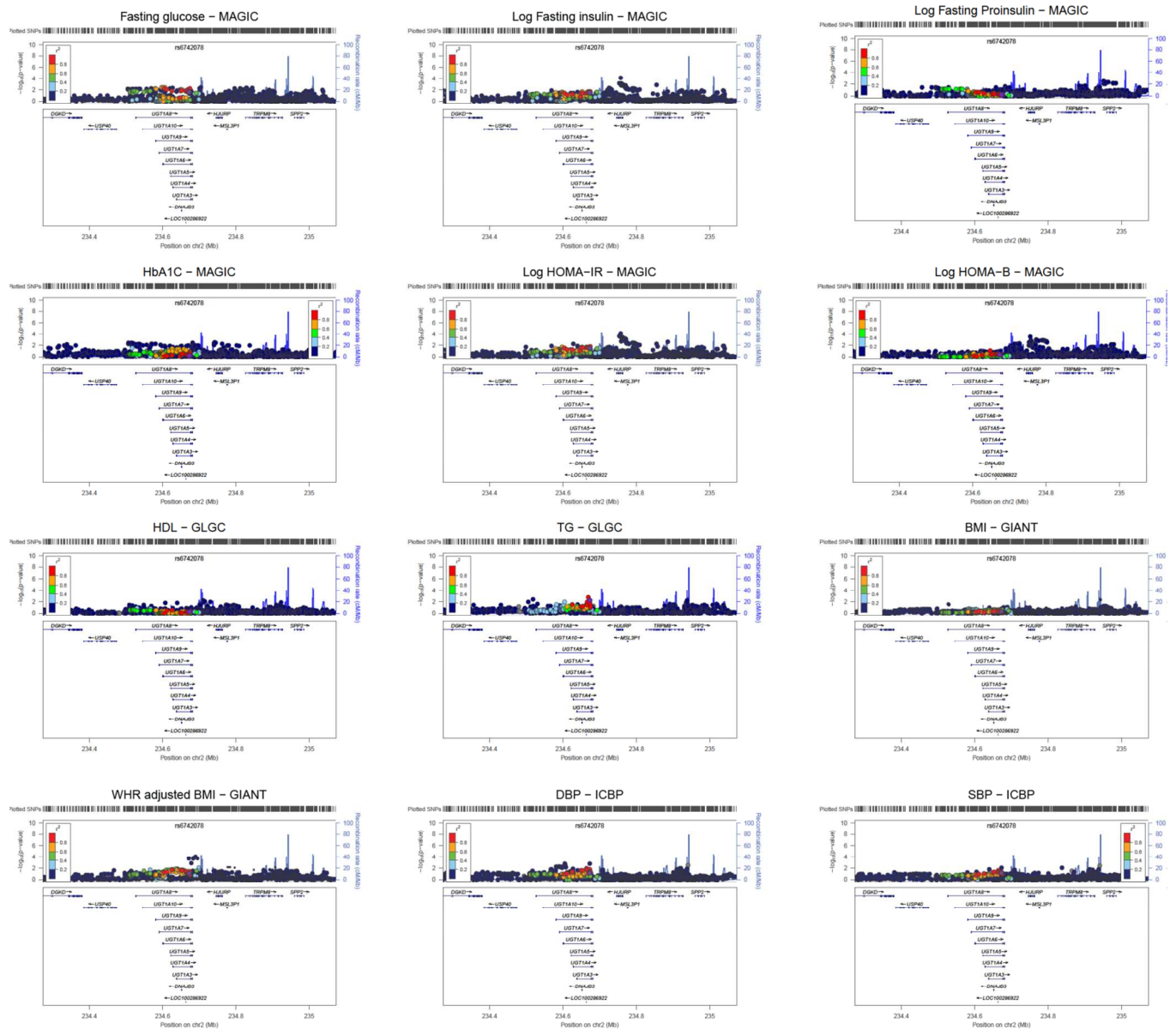
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Figure legends

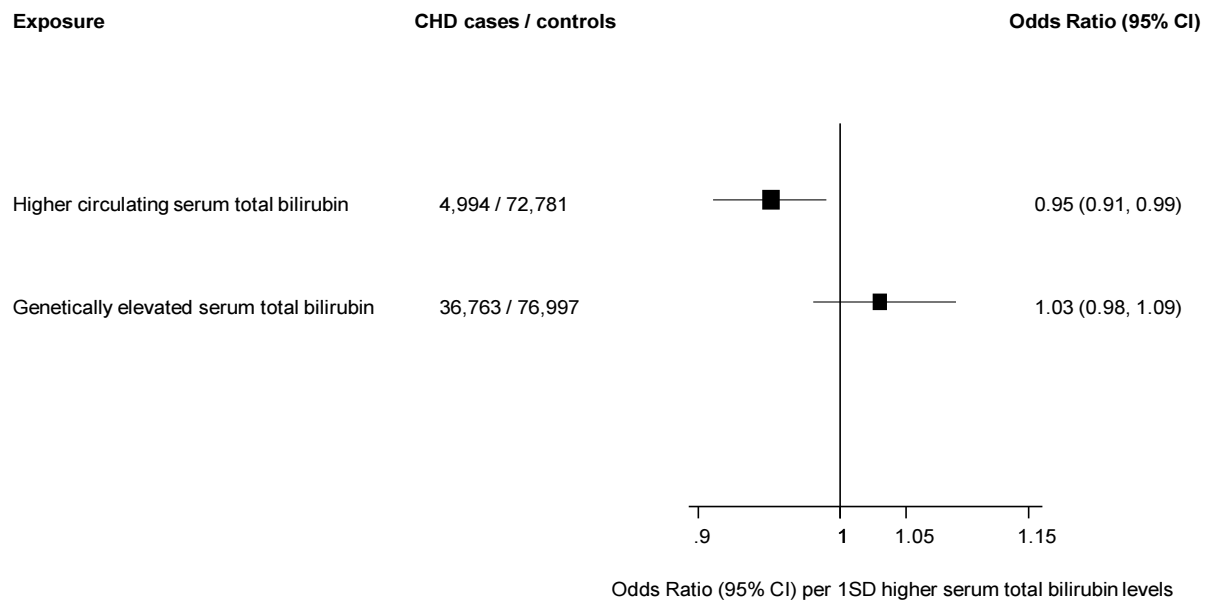
Figure 1. Regional association plots of rs6742078 with cardiometabolic traits



Plots were created with LocusZoom available from <http://csg.sph.umich.edu/locuszoom> using published data from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC); Global Lipids Genetics Consortium (GLGC); Genetic Investigation of ANthropometric Traits (GIANT); and the International Consortium for Blood Pressure (ICBP).

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin ; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; SBP, systolic blood pressures; TG, triglycerides; WHR, waist-to-hip ratio

Figure 2. Comparison of the associations of circulating and genetically elevated serum total bilirubin levels with coronary heart disease



CHD, coronary heart disease; CI, confidence interval; SD, standard deviation

Table 1. Table of the rs6742078 variant or proxies confirmed to be associated with serum total bilirubin levels at genome-wide significance in published studies

GWAS study (reference)	Population	Sample (N)	Lead SNP	Effect allele or minor allele	Effect allele frequency	Variance explained %	beta (SE) or effect (95% CI) per copy effect allele [unless otherwise specified]	P-value for association with total bilirubin
Johnson et al., 2009 (1)	Europeans	9,464	rs6742078	T	0.32	18.0	0.234 (0.005)†	< 5.0E-324
Sanna et al., 2009 (2)	Sardinians	4,300	rs887829	T	0.30	14.3	0.57 (0.032)*	6.21E-62
Chen et al., 2012 (3)	African-Americans	619	rs887829	T	0.45	12.4	0.226 (0.183, 0.270)	1.97E-22
Bielinski et al., 2011 (4)	North-American	6,307	rs4148325	T	0.33	18	0.17 (0.0098)†	4.96E-62
Dai et al., 2013 (5)	Han-Chinese	10,282	rs6742078	A	0.12	4.7	0.153 (0.008)†	1.44E-89

†, beta or effect size represents change in log transformed bilirubin per copy effect allele; *, change in standard deviation units

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