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Serum total bilirubin levels and coronary heart disease — Causal association or epiphenomenon?

Setor K. Kunutsor

School of Clinical Sciences, University of Bristol, Learning & Research Building (Level 1), Southmead Hospital, Southmead Road, Bristol BS10 5NB, UK

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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.

1. 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 (Horsfall et al., 2012)). 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 (Kunutsor et al., 2015). 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 (Keavney, 2002; Petitti and Freedman, 2005). 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" (Davey Smith and Ebrahim, 2003)).

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 (Kronenberg et al., 2002). 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 (Bosma et al., 2003; Lin et al., 2006). There are suggestions that this TA repeat variant might be the key polymorphism within the *UGT1A1* gene controlling serum bilirubin levels (Lin et al., 2009). It has been hypothesized that the *UGT1A1*28* allele may be a protective factor against CHD, which may provide support for a causal

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.

E-mail address: skk31@cantab.net.

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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 (Bosma et al., 2003; Lin et al., 2006). The rs6742078 variant in the *UGT1A1* gene [which has been shown to be in strong linkage disequilibrium with the *UGT1A1*28* allele (Johnson et al., 2009)] 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 (Stender et al., 2013a)) and has been used as an instrument for examining the causal relevance of serum total bilirubin to disease outcomes (Stender et al., 2013a, 2013b). Stender et al. (2013b)) 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 utilizing a MR approach.

2. 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 (Stender et al., 2013a, 2013b). 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) (Schunkert et al., 2011) and the Coronary Artery Disease (C4D) Genetics consortium (Coronary Artery Disease Genetics Consortium, 2011). Estimates of power to detect associations with CHD employed Purcell's online power calculation for

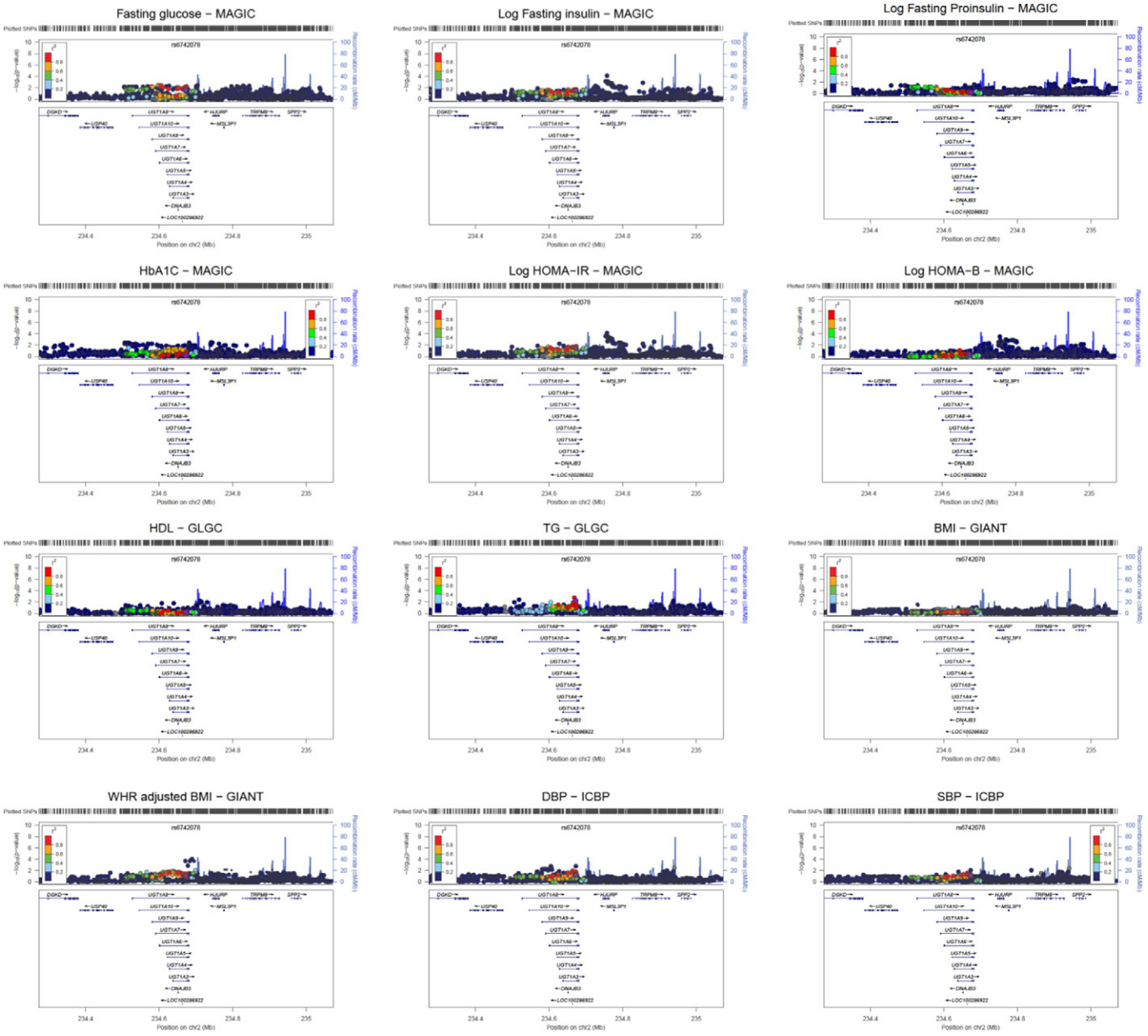

Fig. 1. Regional association plots of rs6742078 with cardiometabolic traits. Plots were created with LocusZoom available from http://csg.sph.umich.edu/locuszoom using published data from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC); Global Lipids Genetics Consortium (GLGC); 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.