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# Genetic determinants of blood lipids and cerebral small vessel disease: role of high-density lipoprotein cholesterol

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Blood lipids are causally involved in the pathogenesis of atherosclerosis, but their role in cerebral small vessel disease remains largely elusive. Here, we explored associations of genetic determinants of blood lipid levels, lipoprotein particle components, and targets for lipid-modifying drugs with small vessel disease phenotypes. We selected genetic instruments for blood levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides, for cholesterol and triglycerides components of size-defined lipoprotein particles, and for lipid-modifying drug targets based on published genome-wide association studies (up to 617 303 individuals). Applying two-sample Mendelian randomization approaches we investigated associations with ischaemic and haemorrhagic manifestations of small vessel disease [small vessel stroke: 11 710 cases, 287 067 controls; white matter hyperintensities (WMH): 10 597 individuals; intracerebral haemorrhage: 1545 cases, 1481 controls]. We applied the inverse-variance weighted method and multivariable Mendelian randomization as our main analytical approaches. Genetic predisposition to higher HDL-C levels was associated with lower risk of small vessel stroke [odds ratio (OR) per standard deviation = 0.85, 95% confidence interval (CI) = 0.78–0.92] and lower WMH volume (β = –0.07, 95% CI = –0.12 to –0.02), which in multivariable Mendelian randomization remained stable after adjustments for LDL-C and triglycerides. In analyses of lipoprotein particle components by size, we found these effects to be specific for cholesterol concentration in medium-sized high-density lipoprotein, and not large or extra-large high-density lipoprotein particles. Association estimates for intracerebral haemorrhage were negatively correlated with those for small vessel stroke and WMH volume across all lipid traits and lipoprotein particle components. HDL-C raising genetic variants in the gene locus of the target of CETP inhibitors were associated with lower risk of small vessel stroke (OR: 0.82, 95% CI = 0.75–0.89) and lower WMH volume (β = –0.08, 95% CI = –0.13 to –0.02), but a higher risk of intracerebral haemorrhage (OR: 1.64, 95% CI = 1.26–2.13). Genetic predisposition to higher HDL-C, specifically to cholesterol in medium-sized high-density lipoprotein particles, is associated with both a lower risk of small vessel stroke and lower WMH volume. These analyses indicate that HDL-C raising strategies could be considered for the prevention of ischaemic small vessel disease but the net benefit of such an approach would need to be tested in a randomized controlled trial.

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**Abbreviations:** GLGC = Global Lipids Genetics Consortium; GWAS = genome-wide association study; HDL-C = high-density lipoprotein cholesterol; ICH = intracerebral haemorrhage; LDL-C = low-density lipoprotein cholesterol; SVD = small vessel disease; SVS = small vessel stroke; WMH = white matter hyperintensities

# Introduction

Cerebral small vessel disease (SVD) accounts for ~20% of all ischaemic strokes (Sudlow and Warlow, 1997) and most cases of intracerebral haemorrhage (ICH) (Qureshi *et al.*, 2001, 2009). SVD is the leading cause of vascular dementia (O'Brien and Thomas, 2015; Iadecola *et al.*, 2019) and an independent predictor of mortality (Debette *et al.*, 2019; Georgakis *et al.*, 2019). Manifestations of SVD on MRI are highly prevalent in the ageing population with figures reaching 90% for white matter hyperintensities (WMH) in patients aged 65 years and above (de Leeuw *et al.*, 2001; Pantoni, 2010; Wardlaw *et al.*, 2019). However, the mechanisms underlying SVD are poorly understood, thus impeding the development of effective strategies for prevention.

Blood lipids are a well-established risk factor for large artery atherosclerosis (Collins *et al.*, 2016) and lipid-modifying therapies have shown benefits in reducing risk of both coronary artery disease and stroke [Cholesterol Treatment Trialists' (CTT) Collaboration *et al.*, 2010; Chou *et al.*, 2016]. Yet, their role in SVD remains largely elusive. Current guidelines for secondary stroke prevention recommend treatment with statins after ischaemic stroke or transient ischaemic attack [European Stroke Organisation (ESO) Executive Committee and ESO Writing Committee, 2008; Kernan *et al.*, 2014; Intercollegiate Stroke Working Party, 2016; Stroke Foundation, 2017) referring to clinical trials data and meta-analyses (Amarenco *et al.*, 2006; Amarenco and Labreuche, 2009; Manktelow and Potter, 2009). However, most trials provided no sub-analyses for ischaemic stroke subtypes. The J-STARS trial, the only study providing sub-analyses, found statins to reduce recurrence of large artery stroke but not small vessel stroke (SVS) (Hosomi *et al.*, 2015). Results from the SPARCL trial suggest that statins may increase the risk of ICH in patients with stroke or transient ischaemic attack (Amarenco *et al.*, 2006), especially in patients with SVS as an entry event (Goldstein *et al.*, 2008).

Mendelian randomization makes use of genetic variants that are associated with an exposure or risk factor as instruments, and investigates their associations with disease outcomes thus overcoming some of the key limitations of observational studies such as confounding and reverse causation (Hopewell and Clarke, 2016; Holmes *et al.*, 2017). Hence, Mendelian randomization analyses can assess the causal relevance of a risk factor for disease and facilitate prioritization of interventions to be tested in clinical trials (Holmes *et al.*, 2017; O'Donnell and Sabatine, 2018) as has specifically been demonstrated for lipid-modifying drugs (Khera and Kathiresan, 2017; Ference *et al.*, 2018). In fact, there are several examples where Mendelian randomization studies have predicted the success or failure of clinical trials (Ference *et al.*, 2015, 2016, 2017*b*, 2019*b*; Gill *et al.*, 2019; Ray *et al.*, 2019). The availability of large scale genome-wide association studies (GWAS) for an expanding range of phenotypes and the development of two-sample Mendelian randomization approaches enable the exploration of associations for which there is a paucity of evidence from clinical trials, as is the case for lipids and cerebral SVD.

Here, we leveraged data from the largest GWAS currently available on blood lipid levels (617 303 individuals) (Willer *et al.*, 2013; Klarin *et al.*, 2018) and on both ischaemic (SVS, WMH volume) and haemorrhagic (ICH) manifestations of cerebral SVD (Woo *et al.*, 2014; Malik *et al.*, 2018*a*; Rutten-Jacobs *et al.*, 2018) with the aim to: (i) examine the effects of genetic determinants of blood levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides on SVD manifestations; (ii) explore associations between genetic determinants of size-defined lipoprotein particle fractions with these phenotypes; and (iii) determine the effects of genetic predisposition to HDL-C raising, LDL-C lowering, and triglyceride lowering through variants in genes encoding targets of lipid-modifying drugs on SVD manifestations.

# Materials and methods

This study follows the guidelines for strengthening the reporting of Mendelian randomization studies (STROBE-MR)

(Davey Smith *et al.*, 2019). We applied two-sample Mendelian randomization analyses, which allow selection of genetic variants as instruments for a risk factor (blood lipid traits) in one sample and explore associations of theses variants with outcomes (manifestations of SVD) in another sample (Davey Smith and Hemani, 2014; Burgess *et al.*, 2015). By overcoming the requirement for assessing the exposure and outcome in the same dataset, this approach enables the exploration of associations in publicly available summary statistics from large GWASs with a corresponding increase in power. Also, two-sample Mendelian randomization is less prone to the winner's curse bias than one-sample Mendelian randomization (Davey Smith and Hemani, 2014; Taylor *et al.*, 2014).

## Study design and data sources

The data sources used for this study are detailed in Supplementary Table 1. In Mendelian randomization analyses, we examined associations of blood lipid levels, size-defined lipoprotein particle fractions, and lipid-modifying drug targets, with ischaemic and haemorrhagic SVD phenotypes. We selected genetic instruments from the GWAS summary statistics of the Million Veteran Program (MVP) (Klarin *et al.*, 2018), the Global Lipids Genetics Consortium (GLGC) (Willer *et al.*, 2013), and from a GWAS on nuclear magnetic resonance (NMR) measured circulating metabolites (Kettunen *et al.*, 2016). We then examined associations of the selected instruments with SVS in the GWAS summary statistics of the MEGASTROKE Consortium (Malik *et al.*, 2018*a*), with WMH volume in a GWAS analysis that we undertook in the UK Biobank neuroimaging dataset (Alfaro-Almagro *et al.*, 2018), and with ICH in the International Stroke Genetics Consortium (ISGC) GWAS meta-analysis (Woo *et al.*, 2014).

## Genetic instrument selection

### Blood lipid levels

We selected genetic instruments for the blood levels of HDL-C, LDL-C, and triglycerides, based on the results of the GWAS multi-ethnic meta-analysis of the MVP and the GLGC samples (617 303 individuals) (Klarin *et al.*, 2018). Specifically, we used independent genetic variants that reached genome-wide level of significance ($P < 5 \times 10^{-8}$) for their associations with HDL-C, LDL-C and triglycerides, in the conditional GWAS meta-analyses as instruments. We identified 312 instruments for HDL-C, 219 for LDL-C, and 253 for triglycerides (Supplementary Table 2). In our primary analyses, we weighted the instruments based on the joint regression coefficients from the conditional GWAS meta-analysis of MVP and GLGC. As the GLGC further excluded participants on lipid-lowering treatment (Willer *et al.*, 2013), to exclude sources of biases related to treatment-mediated effects on blood lipids in the MVP dataset, we performed sensitivity analyses weighting the instruments using the GLGC effect sizes only. Both MVP and GLGC were imputed to the 1000 Genomes Project (Phase 3 and Phase 1, respectively) (1000 Genomes Project Consortium *et al.*, 2012) and included adjustments for age, age$^2$, sex, and population structure.

In a secondary approach, we restricted our selection of instruments to HDL-C-, LDL-C-, and triglyceride-specific variants. In particular, we used the GLGC dataset (188 577 individuals), for which we had access to the full GWAS summary statistics (Willer *et al.*, 2013), and identified those independent genetic variants associated with HDL-C, LDL-C, or triglycerides at genome-wide significance ($P < 5 \times 10^{-8}$), but showed associations of $P > 0.01$ with the other two traits. We found 19 HLD-C-specific, 25 LDL-C-specific, and four triglyceride-specific variants (Supplementary Table 3) and performed sensitivity analyses using them as instruments.

### Size-defined lipoprotein particle fractions

We then selected genetic instruments for cholesterol and triglyceride concentrations in size-defined lipoprotein particles available from a GWAS for NMR-measured circulating metabolites on 24 925 European individuals (Kettunen *et al.*, 2016). The GWAS analyses were imputed to the 1000 Genomes Project (Phase 1) and adjusted for age, sex, time from last meal, and population structure (Kettunen *et al.*, 2016). Based on summary statistics for each trait, we extracted variants after clumping for linkage disequilibrium (LD) at $r^2 < 0.1$ that reached genome-wide significance ($P < 5 \times 10^{-8}$). The identified instruments for each metabolite are available in Supplementary Table 4.

### Variants in genes encoding known lipid-modifying drug targets

Next, we selected variants clumped for linkage disequilibrium at $r^2 < 0.1$ within a region of 100 kb upstream or downstream from genes encoding known drug targets that were associated with the respective lipid trait at a genome-wide significant level ($P < 5 \times 10^{-8}$) in the GLGC dataset (Willer *et al.*, 2013). Specifically, we searched for genetic variants in the *CETP* locus (encoding the target of CETP inhibitors) associated with HDL-C levels; variants in the loci of *HMGCR* (target of statins), *NPC1L1* (target of ezetimibe), *PCSK9* (target of PCSK9 inhibitors), *ABCG5* and *ABCG8* (targets of bile acid resins), and *LDLR* (therapeutic target of the LDL receptor) associated with LDL-C levels; and variants in the *PPARA* locus (target for fibrates) associated with triglyceride levels, in accordance with similar approaches applied by other studies (Ference *et al.*, 2012, 2015, 2017*b*, 2019*a*; Anderson *et al.*, 2016; Harrison *et al.*, 2018; Nowak and Arnlov, 2018). We identified 24 HDL-C raising variants in *CETP*, and for LDL-C lowering targets, four variants in *HMGCR*, three in *NPC1L1*, 11 in *PCSK9*, six in *ABCG5/G8*, and eight in *LDLR* (Supplementary Table 5). No triglyceride-lowering variants were identified in the *PPARA* locus based on our selection criteria for instruments.

For each genetic instrument, we estimated the proportion of variance explained for the respective phenotype and measured instrument strength with *F*-statistics (Supplementary Tables 2–5). *F* was >10 for all selected instruments, indicating a low probability for weak instrument bias (Palmer *et al.*, 2012). Furthermore, we performed power calculations (Burgess, 2014) to identify the range of association estimates that we had >80% power (1 − β) to detect at $\alpha = 0.05$ (Supplementary Table 6).

## Associations with outcomes

The outcomes examined in this study were ischaemic and haemorrhagic manifestations of SVD including SVS, WMH

volume, and ICH. Genetic association estimates for SVS—defined according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria (Adams *et al.*, 1993)—were obtained from the MEGASTROKE multi-ethnic GWAS meta-analysis (Malik *et al.*, 2018*a*, *b*) on 11 710 cases and 287 067 controls. For WMH volume, we performed a GWAS analysis in the UK Biobank Imaging dataset (10 597 individuals of White-British ancestry), based on the measurements of WMH volume in $T_1$ and $T_2$ FLAIR MRI sequences, as previously described, following adjustments for age, sex, and the first 10 principal components (Rutten-Jacobs *et al.*, 2018). We further examined ICH, as well as ICH subtypes defined according to haemorrhage location (deep and lobar). We used summary statistics from the ISGC GWAS meta-analysis including 1545 cases of spontaneous ICH defined by acute neurological onset and compatible neuroimaging showing intraparenchymal haemorrhage (664 lobar, 881 deep) and 1481 controls of European ancestry (Woo *et al.*, 2014).

## Statistical analysis

### Main analyses

We applied two-sample Mendelian randomization analyses based on association estimates derived from the abovementioned sources. Following extraction of the association estimates between the instruments and the outcomes and harmonization of the direction of estimates by effect alleles, we computed Mendelian randomization estimates for each instrument with the Wald estimator and standard errors with the Delta method. All Mendelian randomization estimates were scaled to 1 – SD (standard deviation) increment in the lipid levels or the lipoprotein particle fractions. We then pooled individual Mendelian randomization estimates using random-effects inverse-variance weighted (IVW) meta-analyses. IVW is the most widely used main method for Mendelian randomization analysis because it provides robust causal estimates under absence of directional pleiotropy (Burgess *et al.*, 2013).

Given the correlation between HDL-C, LDL-C, and triglyceride levels, and between cholesterol and triglyceride concentrations in specific size-defined lipoprotein particles, we further performed multivariable Mendelian randomization to disentangle their independent associations with SVD phenotypes (Burgess and Thompson, 2015). For HDL-C, LDL-C, and triglyceride blood levels, we used the respective instruments and adjusted for their effects on the other two traits from the GLGC dataset. For cholesterol concentration in HDL particles, we combined all unique variants associated with either total HDL-C levels or size-defined HDL cholesterol concentration and adjusted for their effects on blood LDL-C and triglyceride levels. Similarly, for cholesterol concentration in LDL and larger particles, we combined all variants associated with either total LDL-C levels or size-defined LDL and larger particle cholesterol concentrations and adjusted for their effects on HDL-C and triglyceride levels. Finally, we combined instruments for either total circulating triglyceride levels or for particle-specific triglyceride concentrations and adjusted for their effects on HDL-C and LDL-C.

For all analyses, we corrected for multiple comparisons with the false discovery rate (FDR) approach and set statistical significance at a q-value $< 0.05$. Associations not reaching this threshold, but showing a $P < 0.05$, were considered suggestive of an association.

### Assessment of pleiotropy and sensitivity analyses

The IVW method was our primary Mendelian randomization analysis approach, but the derived estimates might be biased in case of directional pleiotropy. As a measure of pleiotropy, we assessed heterogeneity across the Mendelian randomization estimates for each instrument in the IVW Mendelian randomization analyses with the Cochran's Q statistic (Bowden *et al.*, 2018). Under presence of nominal heterogeneity ($P$ from Cochran's $Q < 0.10$) we further applied alternative Mendelian randomization methods, which are more robust to the use of pleiotropic instruments. These were the weighted median estimator and the Mendelian randomization (MR)-Egger regression. The weighted median estimator allows the use of invalid instruments under the assumption that at least half of the instruments used in the Mendelian randomization analysis are valid (Hartwig *et al.*, 2017). The MR-Egger regression allows for the estimation of an intercept term, which can be used as an indicator of unbalanced directional pleiotropy (Bowden *et al.*, 2015). MR-Egger provides less precise estimates and relies on the assumption that the strengths of potential pleiotropic instruments are independent of their direct associations with the outcome (Bowden *et al.*, 2015). The intercept obtained from MR-Egger regression was used as a measure of directional pleiotropy ($P < 0.05$ indicated statistical significance) (Bowden *et al.*, 2015).

In case of evidence of directional pleiotropy (as assessed by both the Cochran's Q statistic and the intercept in the MR-Egger regression) and inconsistent results between the different approaches, we further applied the generalized summary data-based Mendelian randomization (GSMR) approach. This method uses all variants reaching genome-wide significance as instruments by accounting for linkage disequilibrium correlation between them and further identifies and eliminates outliers that exert apparent pleiotropic effects on both the risk factor and the outcome using the HEIDI-outlier method (Zhu *et al.*, 2018). GSMR further provides a measure of remaining global heterogeneity following exclusion of outliers that also takes into account the low linkage disequilibrium across the used instruments.

All analyses were performed in R (v3.5.0; The R Foundation for Statistical Computing) using the MendelianRandomization and the gsmr packages.

## Data availability

The data used for the current study are publicly available and may also become available from the corresponding author on reasonable request.

# Results

## Genetic determinants of blood lipid levels and ischaemic small vessel disease

The primary results of the IVW Mendelian randomization analyses for the associations between genetic determinants of blood lipid levels and SVS and WMH volume are presented in Fig. 1. Genetic predisposition to elevated HDL-C

levels were associated with both a lower risk of SVS [odds ratio (OR): 0.85, 95% CI: 0.78–0.92, $P = 5 \times 10^{-4}$] and lower WMH volume (β: –0.07, 95% CI: –0.12 to –0.02, $P = 0.004$). We further found genetic predisposition to higher triglyceride levels to be associated with higher risk of SVS and a suggestive association between genetic predisposition to higher LDL-C levels and SVS risk. In multivariable Mendelian randomization, the associations between genetic determinants of HDL-C levels and SVS and WMH volume remained stable and statistically significant (Fig. 1). In contrast, the association between genetic determinants of triglyceride levels and SVS was attenuated when adjusting for HDL-C and LDL-C.

The Mendelian randomization results were stable when weighting the genetic instruments for the three lipid traits based on their association estimates in the GLGC dataset, which excluded individuals on lipid-modifying treatment (Supplementary Figs 1 and 2). In Mendelian randomization analyses restricted to the instruments specifically associated with HDL-C, LDL-C, or triglycerides, the association estimates of genetic determinants of HDL-C for both risk of SVS (OR: 0.78, 95% CI: 0.62–0.98) and WMH volume (β: –0.27, 95% CI: –0.45 to –0.08) were even stronger (Supplementary Fig. 3). GSMR-HEIDI, which identifies and excludes pleiotropic outlier variants, also showed significant associations between genetic predisposition to higher HDL-C and both lower SVS risk and lower WMH volume (Supplementary Figs 1 and 2).

## Genetic determinants of size-defined lipoprotein particle fractions and ischaemic small vessel disease

To obtain a deeper understanding of the observed associations, we next selected genetic instruments for cholesterol and triglyceride concentrations in size-defined lipoprotein particles and examined their associations with SVS and WMH volume (Fig. 2 and Supplementary Table 7). We found genetic predisposition to higher cholesterol concentration in the medium-sized, but not in the large- or extra-large sized HDL particles, to be associated with both lower SVS risk (OR: 0.84, 95% CI: 0.73–0.96, $P = 0.007$) and lower WMH volume (β: –0.09, 95% CI: –0.16 to –0.02, $P = 0.009$). There was no heterogeneity and the associations remained significant when adjusting for the effects of the instruments on circulating LDL-C and triglyceride levels (Fig. 2 and Supplementary Table 8).

Because of evidence for heterogeneity (Cochran's Q $P < 0.10$) and inconsistent results for the associations of genetic determinants of total HDL-C with SVS risk and WMH volume across sensitivity analyses (weighted median and MR-Egger) (Fig. 3 and Supplementary Figs 1 and 2), we next restricted the set of instruments for total HDL-C to those associated with medium-sized HDL-C ($P < 5 \times 10^{-8}$). These analyses revealed stronger association estimates between genetic predisposition to higher HDL-C and both lower risk of SVS (OR: 0.69, 95% CI: 0.56–0.84, $P = 4 \times 10^{-4}$) and lower WMH volume (β: –0.23, 95% CI: –0.35 to –0.10, $P = 2 \times 10^{-4}$) (Fig. 3). Moreover, the estimates were highly consistent in alternative Mendelian randomization approaches with no evidence for heterogeneity, thus suggesting that heterogeneity in the overall analyses was driven by non-medium sized HDL-C increasing variants.

To explore whether the observed associations were specific to genetic predisposition to higher cholesterol concentration in the medium-sized HDL, we next expanded our analyses to other components of the HDL particles (Supplementary Fig. 4). In this *post hoc* analysis, we found similar association estimates between genetic determinants of the concentration of any of the medium-sized HDL particle components (total cholesterol, cholesterol-esters, free cholesterol, total lipids, and phospholipids) and SVS risk as well as WMH volume suggesting that the associations are driven by the medium-sized HDL particles as a whole.

Regarding other lipoprotein particle components, we further found genetic predisposition to higher concentration of triglycerides in the small-sized HDL particles to be associated with higher risk of SVS (Fig. 2 and Supplementary Tables 7–9).

## Genetic variants in loci of lipid-modifying drug targets and ischaemic small vessel disease

We next selected genetic variants in genes encoding known HDL-C-raising or LDL-C-lowering drug targets and examined their associations with ischaemic SVD phenotypes. HDL-C-raising variants in the *CETP* locus were associated with lower risk of SVS (OR: 0.82, 95% CI = 0.75–0.89, $P = 9 \times 10^{-6}$) and lower WMH volume (β = –0.08, 95% CI = –0.13 to –0.02, $P = 0.008$) (Figs 4, 5A and B). While there was heterogeneity in the association between *CETP* variants and SVS ($P = 0.03$), the results remained significant in the weighted median and MR-Egger approaches (Supplementary Table 10). As previous analyses from the REVEAL trial (HPS3/TIMI55–REVEAL Collaborative Group *et al.*, 2017) had shown the beneficial effects of cholesteryl-ester transfer protein (CETP) inhibitors on vascular disease to be mainly driven by their LDL-C lowering and not their HDL-C raising capacity, we further explored the associations between genetic predisposition to LDL-C lowering through *CETP* variants and ischaemic SVD manifestations. While genetic predisposition to LDL-C lowering was associated with lower risk of SVS and lower WMH volume in univariable IVW Mendelian randomization analyses, these effects were entirely reversed after adjusting for the HDL-C raising effects of the variants in multivariable Mendelian randomization (Supplementary Table 11). Analyses for genetic variants in LDL-C lowering drug target loci showed no statistically significant results (Fig. 4).

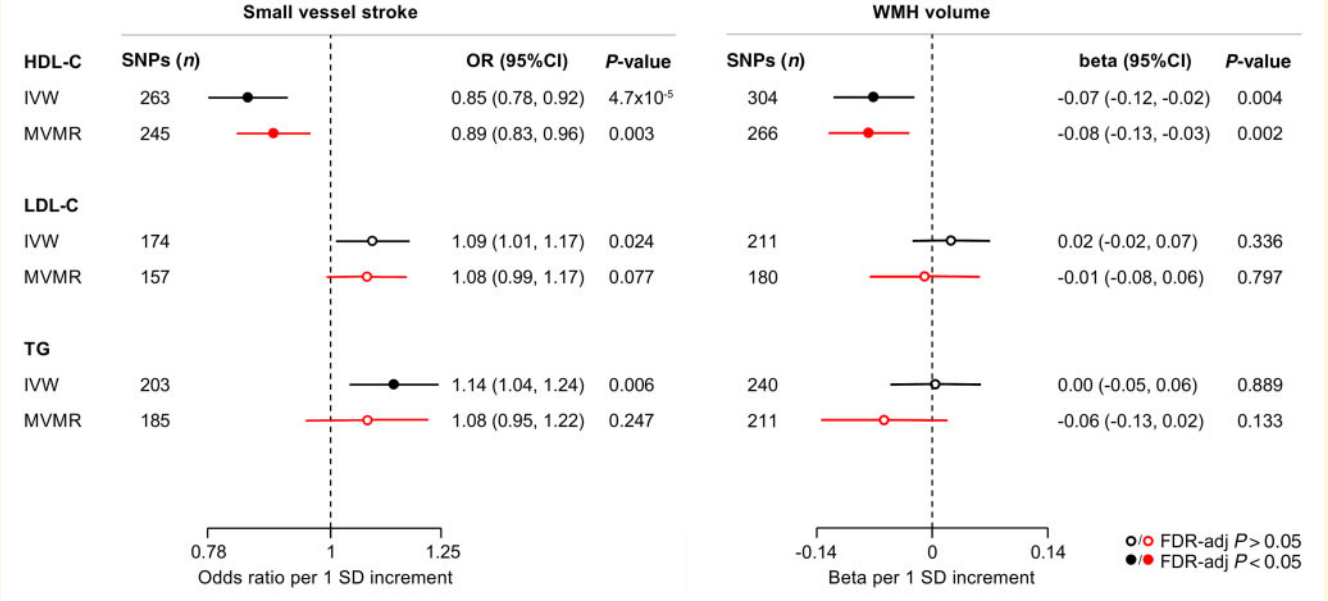


**Figure 1 Mendelian randomization associations of genetic determinants of blood lipid levels (HDL-C, LDL-C, triglycerides) with risk of small vessel stroke and WMH volume.** Shown are the results derived from random-effects IVW (inverse-variance weighted) Mendelian randomization and multivariable Mendelian randomization (MVMR) analyses adjusting for the effects of the genetic variants on all the three blood lipid traits. SD = standard deviation; TG = triglycerides.

## Genetic associations of lipid traits with intracerebral haemorrhage

IVW-Mendelian randomization analyses showed no significant associations of genetic determinants of HDL-C, LDL-C, and triglycerides with risk of ICH (Fig. 6). When examining lipoprotein particle fractions, we found associations of the opposite direction, as compared to both SVS and WMH volume (Fig. 7). However, confidence intervals were wide, likely due to lack of statistical power (Supplementary Fig. 5 and Supplementary Tables 6 and 12). Across drug target loci (Supplementary Fig. 6) we found HDL-C raising variants in the *CETP* locus to be associated with a higher risk of ICH (OR: 1.64, 95% CI: 1.26–2.13, $P = 2.6 \times 10^{-4}$) (Fig. 5C). This effect was significant for both deep (OR: 2.01, 95% CI: 1.27–3.18, $P = 0.003$) and lobar ICH (OR: 1.78, 95% CI: 1.06–2.89, $P = 0.028$) (Supplementary Fig. 7).

# Discussion

The main findings from this study can be summarized as follows: (i) we found significant associations between genetic predisposition to higher HDL-C levels and both lower risk of SVS and lower WMH volume; (ii) associations were specific for cholesterol concentrations in the medium and not large or extra-large sized HDL particles; (iii) exploring genetic variants at loci for targets of lipid-modifying drugs, we found HDL-C raising variants in *CETP* to be associated with a lower SVS risk and lower WMH volume; and (iv) we found these HDL-C raising variants in *CETP* to be associated with a higher risk of ICH, with consistent results for both lobar and deep ICH.

Our Mendelian randomization results provide evidence for a protective role of HDL-C on ischaemic SVD. This agrees with findings from two small observational studies. In the Women's Healthy Ageing Project, midlife HDL-C levels among 135 females were inversely associated with WMH volume after 20 years, independently of other vascular risk factors (Aljondi *et al.*, 2018). Similarly, in a cross-sectional study of 817 participants aged $\geqslant 50$ years, higher HDL-C levels were associated with lower volumes of both deep and periventricular WMH after adjusting for vascular risk factors (Yin *et al.*, 2018). The mechanisms underlying the observed inverse association between HDL-C levels and ischaemic SVD are unknown but may involve protective effects on the vascular endothelium (Sorrentino *et al.*, 2010; Prosser *et al.*, 2012; Tran-Dinh *et al.*, 2013; Monette *et al.*, 2016). Endothelial cells, including those of the brain microvasculature (Lapergue *et al.*, 2010; Fung *et al.*, 2017), express receptors, which upon HDL binding, induce intracellular signalling eventually leading to vasodilatory (Yuhanna *et al.*, 2001; Spieker *et al.*, 2002; Nofer *et al.*, 2004), anti-inflammatory (Cockerill *et al.*, 1995; Nicholls *et al.*, 2005; Murphy *et al.*, 2008), antioxidative (Garner *et al.*, 1998; Lee *et al.*, 2005; Terasaka *et al.*, 2007), and anti-thrombotic effects (Viswambharan *et al.*, 2004; Calkin *et al.*, 2009).

Our findings contrast with Mendelian randomization analyses on atherosclerotic phenotypes supporting no association of genetic determinants of HDL-C levels with

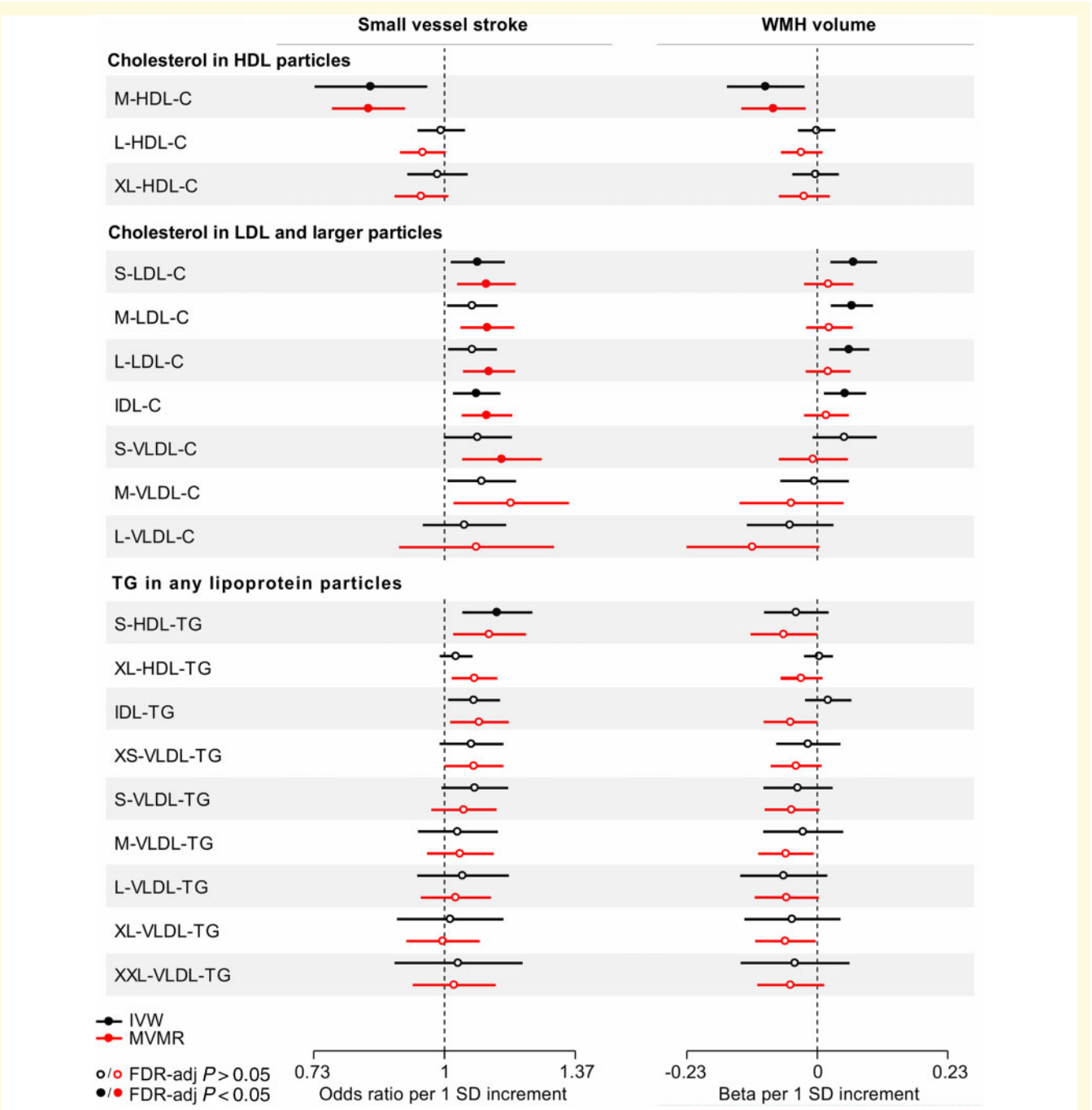


**Figure 2 Mendelian randomization associations of genetic determinants of cholesterol (C) and triglyceride (TG) concentrations in size-defined lipoprotein particles with risk of small vessel stroke and WMH volume.** Shown are the results derived from random-effects inverse-variance weighted (IVW) Mendelian randomization and multivariable Mendelian randomization (MVMR) analyses. MVMR for cholesterol in HDL particles adjusted for LDL-C and triglycerides; for cholesterol in LDL and larger particles adjusted for HDL-C and triglycerides; and for triglycerides in any particles adjusted for HDL-C and LDL-C. IDL = intermediate density lipoprotein; L = large; M = medium; S = small; VLDL = very low density lipoprotein; XL = extra-large.

coronary artery disease (Voight *et al.*, 2012; Holmes *et al.*, 2015; White *et al.*, 2016) and large artery stroke (Hindy *et al.*, 2018) thus suggesting differential effects of HDL-C on cerebral SVD and large artery atherosclerosis. A disparity in the effect of lipid levels between small and large vessel pathologies has also been reported for LDL-C: previous Mendelian randomization studies found strong effects of genetic predisposition to higher LDL-C on the risk of coronary artery disease, large artery stroke, and peripheral artery disease (Holmes *et al.*, 2015; Hindy *et al.*, 2018; Valdes-Marquez *et al.*, 2019; Emanuelsson *et al.*, 2019), but no effect on risk of retinopathy and neuropathy,

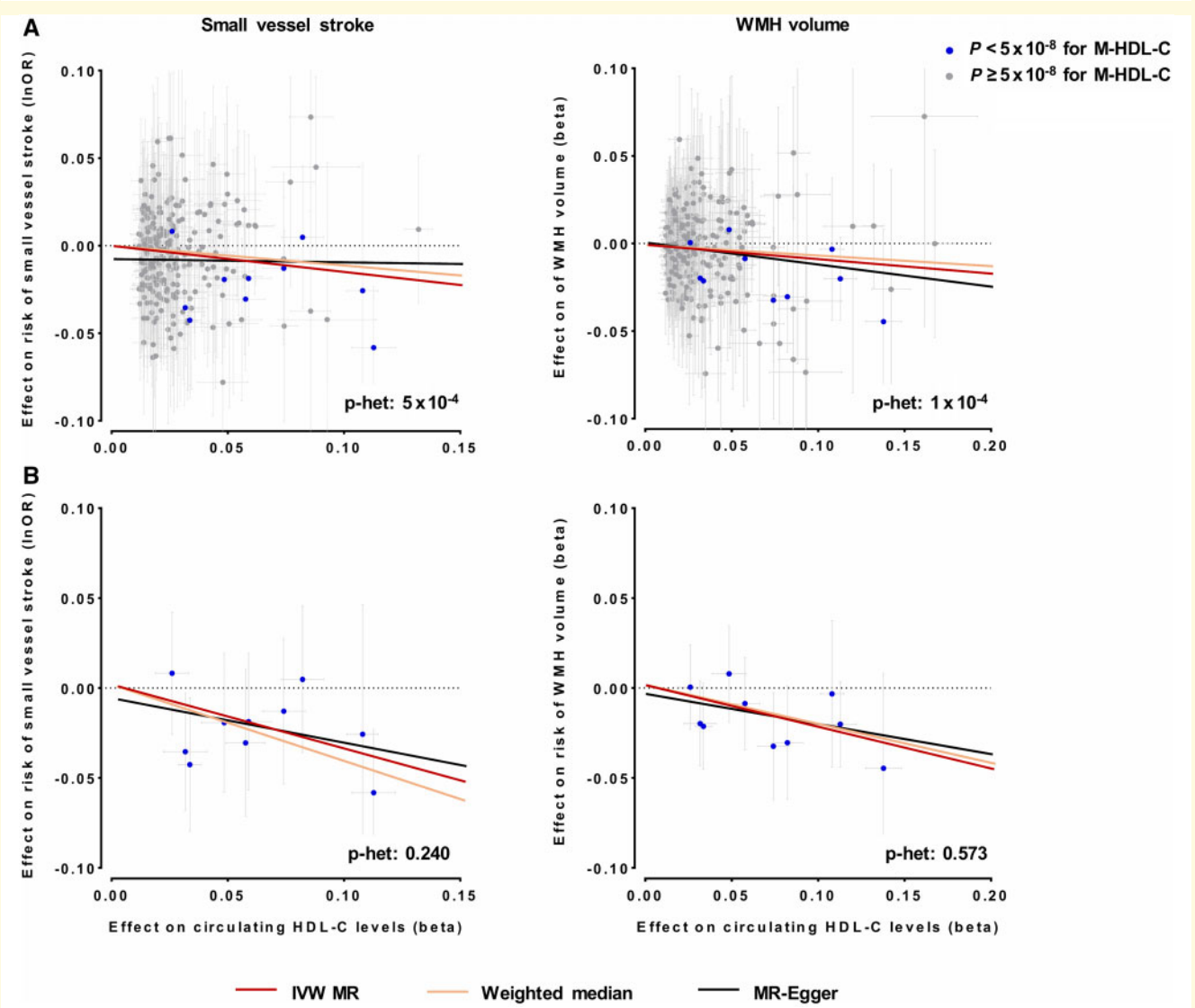


**Figure 3 Mendelian randomization (MR) associations of genetic determinants of HDL-C with risk of small vessel stroke and WMH volume.** Shown are the results from random-effects inverse-variance weighted (IVW), weighted median and MR-Egger analyses when (**A**) using the full set of genetic instruments and (**B**) restricting the analyses to instruments also associated with cholesterol concentration in medium-sized HDL.

which are typically related to small vessel pathology (Emanuelsson *et al.*, 2019). Future studies should explore potentially distinct mechanisms through which blood lipids influence the risk of small versus large vessel disease.

Analysing size-defined lipoprotein particle subfractions we found that the protective effects of HDL-C on ischaemic SVD are specific for medium-sized, and not larger HDL particles. In additional analyses, this effect seemed to be not specific to a particular component of the HDL particles but rather uniform across the different components, thus suggesting that medium-sized HDL particles as a whole could underlie this observation. HDL comprises a heterogeneous pool of lipoprotein particles (Kontush and Chapman, 2010) and the few observational studies that have performed analyses stratified by particle size indeed found differential effects on vascular outcomes (Martin *et al.*, 2015; Wurtz *et al.*, 2015; Joshi *et al.*, 2016; Holmes *et al.*, 2018). There are technical challenges related to different methods of HDL subfractioning (Superko *et al.*, 2012), making it challenging to compare our results with those from previous studies. Still, our results agree with the general notion that the favourable effects observed for HDL are predominantly exerted by the smaller and denser HDL particles (Yu *et al.*, 2003; Williams, 2012; Martin *et al.*, 2014). Of note, previous Mendelian randomization studies on blood lipids that showed no significant associations between HDL-C levels and atherosclerotic phenotypes did not consider particle subfractions (Holmes *et al.*, 2015; White *et al.*, 2016; Hindy *et al.*, 2018). Conceivably, disregarding subfractions might result in

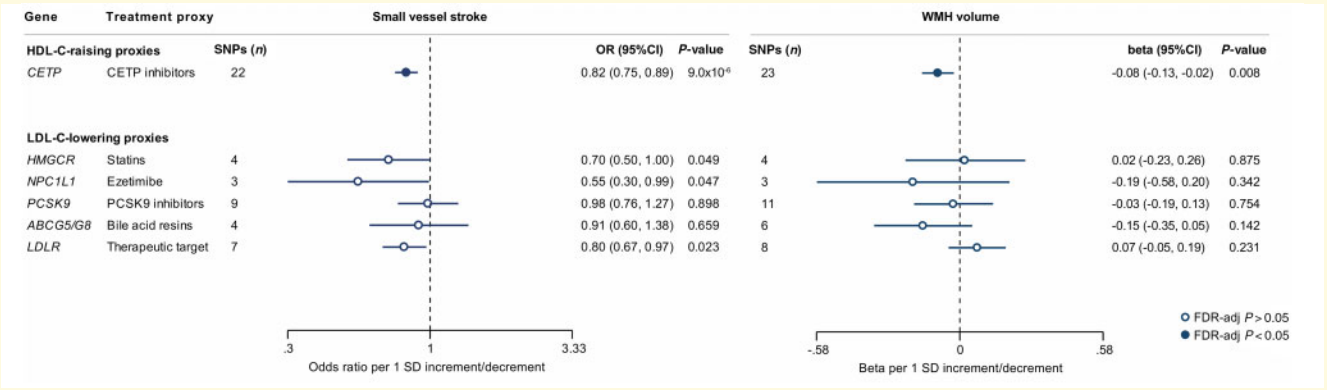


**Figure 4 Mendelian randomization associations of HDL-C-raising and LDL-C-lowering genetic variants in the loci of known lipid-modifying drug targets with risk of SVS and WMH volume.** Shown are the results derived from random-effects inverse-variance weighted (IVW) Mendelian randomization analyses. The results are scaled per 1 SD increment in circulating HDL-C levels (HDL-C-raising drug targets) and per 1 SD increment in circulating LDL-C levels (LDL-C-lowering drug targets).

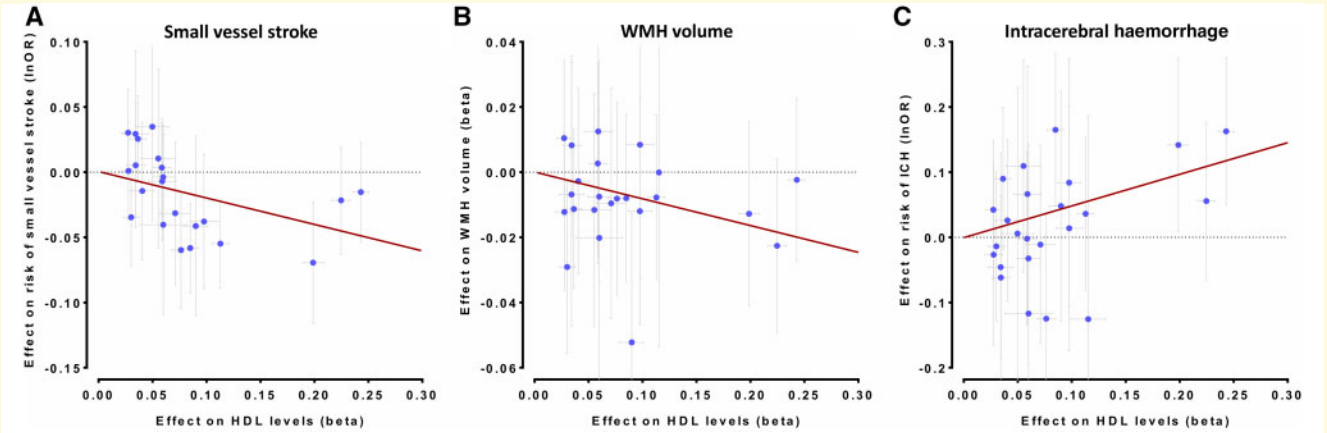


**Figure 5 Mendelian randomization associations between HDL-C raising genetic variants in the *CETP* locus and (A) risk of SVS, (B) WMH volume, and (C) risk of ICH.** Shown are the results from the random-effects inverse-variance weighted (IVW) Mendelian randomization approach. The results are scaled per 1 SD increment in circulating HDL-C levels.

masking causal effects of potential biological relevance. As such, our findings highlight the importance of sub-analyses stratifying by lipoprotein particle size, but the complexity of the potential underlying mechanisms necessitates further study of our observations.

Importantly, we found HDL-C raising genetic variants in the *CETP* locus to also associate with lower SVS risk and WMH volume. Pharmacological CETP inhibition leads to an increase in the circulating pool of HDL particles (Armitage *et al.*, 2019). While initial randomized trials investigating CETP inhibitors on top of statins found no benefit of CETP inhibition on vascular risk (Barter *et al.*, 2007; Schwartz *et al.*, 2012; Lincoff *et al.*, 2017), the most recent REVEAL trial showed a reduced risk for major coronary events (HPS3/TIMI55–REVEAL Collaborative Group *et al.*, 2017). In light of the relatively small effect (relative risk reduction in REVEAL: 9%) it seems unlikely that CETP inhibitors will achieve approval for prevention of cardiovascular disease (Hegele, 2017; Badimon, 2018). However, none of these trials explicitly reported effects on risk of SVS or other SVD manifestations. Our Mendelian randomization results suggest that *post hoc* analyses should consider stratifying for stroke subtypes, and that HDL-C raising approaches might show promise as a strategy for lowering the burden of ischaemic SVD.

The exact mechanism by which CETP inhibition might reduce risk of SVS and WMH volume is poorly understood. In the REVEAL trial, the reduction in vascular risk by CETP inhibition was mediated by a reduction in LDL-C rather than an increase in HDL-C (HPS3/TIMI55–REVEAL Collaborative Group *et al.*, 2017). In our analyses, most of the HDL-C raising *CETP* variants also showed strong associations with lower LDL-C levels. Yet, in multivariable Mendelian randomization analyses adjusting for the effects of the variants on both HDL-C and LDL-C, we found only the effects of genetic predisposition to higher HDL-C

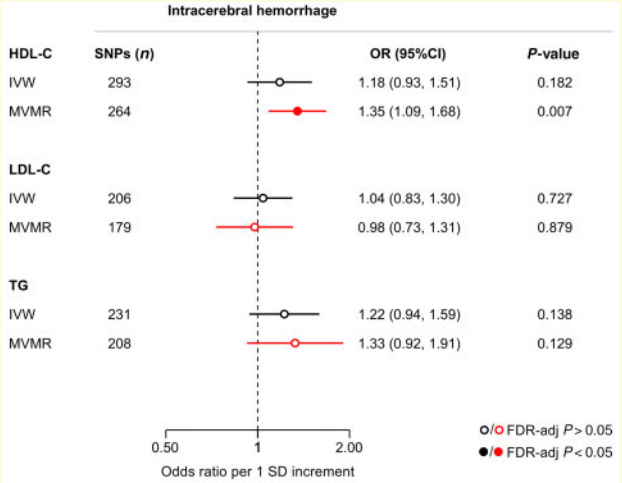


**Figure 6 Mendelian randomization associations of genetic determinants of blood lipid levels (HDL-C, LDL-C, triglycerides) with risk of intracerebral haemorrhage.** Shown are the results derived from random-effects inverse-variance weighted (IVW) Mendelian randomization and multivariable Mendelian randomization (MVMR) analyses. MVMR for cholesterol in HDL particles adjusted for LDL-C and triglycerides; for cholesterol in LDL and larger particles adjusted for HDL-C and triglycerides; and for triglycerides in any particles adjusted for HDL-C and LDL-C. TG = triglycerides.

through these *CETP* variants to remain consistent in terms of magnitude and directionality. Thus, although we were not sufficiently powered to entirely disentangle the effects of the two traits, our results suggest that in contrast with the REVEAL trial results, the effects of *CETP* variants on SVD manifestations might be primarily exerted by HDL-C raising. Administration of CETP inhibitors increases HDL particle size (Brousseau *et al.*, 2004) and genetic predisposition to higher CETP concentration is associated with increased concentrations of medium- and large-sized, but not smaller HDL particles (Blauw *et al.*, 2019). However, whether the expected effects of CETP inhibition on SVS and WMH volume are mediated through increases in the pool of specific HDL subparticles would need to be explored in future studies.

Previous observational and genetic studies found high HDL-C and low LDL-C levels to be associated with a higher risk of ICH (Wang *et al.*, 2013; Anderson *et al.*, 2016; Sun *et al.*, 2019). Also, clinical trials have shown that LDL-C lowering with statins might increase risk for ICH (Amarenco *et al.*, 2006; Goldstein *et al.*, 2008). We found HDL-C raising variants in the *CETP* locus to be associated with a higher risk of both deep and lobar ICH, which relate to different vascular pathologies. Specifically, deep ICH has been associated with hypertensive SVD, whereas lobar ICH is typically related to cerebral amyloid angiopathy (Martini *et al.*, 2012). While speculative, low serum LDL-C and high HDL-C levels may be associated with a fragile vascular endothelium, eventually leading to vessel permeability and a higher susceptibility to rupture (Konishi *et al.*, 1993).

The main analytical approaches used in the current study, IVW and multivariable Mendelian randomization, are sensitive to directional pleiotropy (Lawlor *et al.*, 2008; Burgess and Thompson, 2015). Specifically, if the single nucleotide polymorphisms used as genetic instruments for blood lipid levels associate with manifestations of SVD through pathways independent of blood lipid levels, the results could be biased. To ameliorate this risk, we performed a series of sensitivity analyses, which are based on statistical models that are more robust to pleiotropy, are focused on a subset of genetic instruments that are more specifically associated with the blood lipid traits under study, or excluded outlier single nucleotide polymorphisms with out of average effects on SVD manifestations, which are more likely to exert pleiotropic effects. Importantly, our results for an association between genetic determinants of HDL-C levels with risk of SVS and WMH volume were robust across these sensitivity analyses, thus supporting the results of the main analyses.

Our study has several strengths. The use of large genetic datasets enabled us to explore associations with a range of phenotypes, covering key manifestations of cerebral SVD. Also, the use of GWAS data for NMR-derived measurements enabled analyses stratified for lipoprotein particle subfractions. We further performed multiple tests for the detection of unbalanced pleiotropy and used multiple sensitivity analyses including advanced approaches such as GSMR-HEIDI. These analyses showed consistent results, thus minimizing the possibility of bias in the Mendelian randomization analyses. Finally, we explored the effects of HDL-C raising or LDL-C lowering genetic variants in genes encoding known lipid-modifying drug targets; this approach has previously been validated with the Mendelian randomization effects being comparable to those derived from randomized controlled trials.

Our study also has limitations. First, Mendelian randomization examines the lifetime effect of genetic determinants of blood lipid levels, which might differ from the effects of clinical lipid-modifying interventions. Second, we were not sufficiently powered to identify significant associations for ICH, and especially for ICH subtypes. Similarly, the non-significant, but still suggestive associations between LDL-C levels and SVS risk should be tested in larger datasets offering greater statistical power. Third, we had no access to the full summary statistics from the meta-analysis of the MVP and the GLGC studies. Hence, some analyses were restricted to the smaller GLGC dataset. Fourth, we are not aware of any sufficiently powered GWAS on cerebral microbleeds that would more accurately capture the spectrum of haemorrhagic SVD pathology than the currently used phenotype of ICH. While SVD is an important cause of ICH as a severe clinical manifestation, SVD more frequently manifests with subclinical cerebral microbleeds. Future GWAS on cerebral microbleeds will facilitate Mendelian randomization analyses on the relationship with

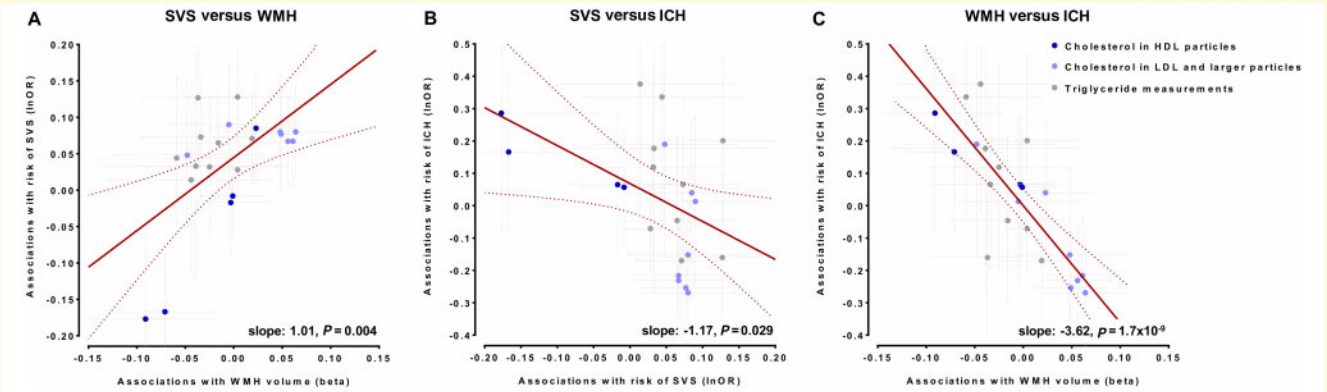


**Figure 7 Comparisons of association estimates for genetic determinants of lipid traits (blood lipid levels and concentrations of lipoprotein particle components) between SVS, WMH volume, and ICH.** Comparisons of the Mendelian randomization association estimates between genetic determinants of lipid traits and risk of SVS with the Mendelian randomization association estimates for WMH volume and risk of ICH. Shown are the meta-regression slopes for the comparisons of these association estimates for: (**A**) risk of SVS and WMH volume, (**B**) risk of SVS and risk of ICH, and (**C**) WMH volume and risk of ICH. Estimates are scaled per 1 SD increment.

blood lipids. Finally, we could not identify valid triglyceride-lowering variants in the locus of the target for fibrates. Hence, we could not explore their associations with SVD phenotypes. Future studies leveraging even larger GWAS datasets on blood lipid levels might identify genetic instruments for the full range of lipid-modifying drug classes.

In conclusion, our results suggest causal associations between higher HDL-C levels and both a lower risk of SVS and lower WMH volume, which were driven by cholesterol concentrations in medium-sized, and not larger HDL particles. HDL-C raising strategies might be of benefit for the prevention of ischaemic SVD. Considering the predicted increase in risk of ICH, the net benefit of such an approach would need to be tested in a randomized controlled trial.

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# Competing interests

C.D.A. receives sponsored research support from the National Institutes of Health of the United States, the American Heart Association, Massachusetts General Hospital, and Bayer AG, and has consulted for ApoPharma, Inc. J.C.H. receives personal fellowship support from the British Heart Foundation [FS/14/55/30806]. J.C.H. works in the Clinical Trial Service Unit & Epidemiological Studies Unit of the Nuffield Department of Population Health at the University of Oxford, which has received research grants from Abbott, AstraZeneca, Bayer, Boehringer Ingelheim, GlaxoSmithKline, The Medicines Company, Merck, Mylan, Novartis, Pfizer, Roche, Schering, and Solvay, which are governed by University of Oxford contracts that protect their independence. In line with the Clinical Trial Service Unit & Epidemiological Studies Unit staff policy, J.C.H. does not take any personal payments directly or indirectly from industry (with reimbursement sought only for the costs of travel and accommodation to attend scientific meetings)

for clinical trial involvement. M.K.G., R.M., K.G.P., and M.D. have no competing interests to declare.

## Supplementary material

Supplementary material is available at *Brain* online.

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