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Differential effects of *PCSK9* variants on risk of coronary disease and ischaemic stroke

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Aims *PCSK9* genetic variants that have large effects on low-density lipoprotein cholesterol (LDL-C) and coronary heart disease (CHD) have prompted the development of therapeutic PCSK9-inhibition. However, there is limited evidence that *PCSK9* variants are associated with ischaemic stroke (IS).

Methods and results Associations of the loss-of-function *PCSK9* genetic variant (rs11591147; R46L), and five additional *PCSK9* variants, with IS and IS subtypes (cardioembolic, large vessel, and small vessel) were estimated in a meta-analysis involving 10 307 IS cases and 19 326 controls of European ancestry. They were then compared with the associations of these variants with LDL-C levels (in up to 172 970 individuals) and CHD (in up to 60 801 CHD cases and 123 504 controls). The rs11591147 T allele was associated with 0.5 mmol/L lower LDL-C level ($P = 9 \times 10^{-143}$) and 23% lower CHD risk [odds ratio (OR): 0.77, 95% confidence interval (CI): 0.69–0.87, $P = 7 \times 10^{-6}$]. However, it was not associated with risk of IS (OR: 1.04, 95% CI: 0.84–1.28, $P = 0.74$) or IS subtypes. Information from additional *PCSK9* variants also indicated consistently weaker effects on IS than on CHD.

Conclusion *PCSK9* genetic variants that confer life-long lower PCSK9 and LDL-C levels appear to have significantly weaker, if any, associations with risk of IS than with risk of CHD. By contrast, similar proportional reductions in risks of IS and CHD have been observed in randomized trials of therapeutic PCSK9-inhibition. These findings have implications for our understanding of when Mendelian randomization can be relied upon to predict the effects of therapeutic interventions.

Keywords PCSK9 • Genetics • LDL-cholesterol • Coronary disease • Stroke • Cardiovascular therapies

Introduction

The causal association between low-density lipoprotein cholesterol (LDL-C) level and coronary heart disease (CHD) risk is well established. However, the observational and randomized evidence for the associations of LDL-C with ischaemic stroke (IS) risk is contrasting. In observational studies, LDL-C is much more weakly associated with IS than with CHD (about 10% vs. 30% lower relative risk per 1 mmol/L lower LDL-C).[1] By contrast, in randomized controlled trials, statin therapy that lowered LDL-C levels for about 5 years has been found to produce similar proportional reductions in the risks of IS and CHD (20–25% per 1 mmol/L LDL-C reduction).[2] Ezetimibe, which lowers LDL-C by a different mechanism to statins, also reduces risk of IS.[3] Whereas observational studies of a risk factor may be prone to confounding and other biases, the specific treatment assessed in a randomized trial may have effects beyond those produced by modifying the particular risk factor.

Genetic instruments that produce life-long exposure to a risk factor may help to determine whether associations with particular health

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outcomes are causal, and can also be used to elucidate the effects of biological mechanisms that are akin to therapeutic interventions. This 'Mendelian randomization' approach can avoid the difficulties in interpretation due to potential biases in observational studies and lack of specificity in randomized trials. However, to be able to detect or refute relevant effects, it requires the availability of genetic variants that produce sufficiently large effects on the risk factor (without material effects on other factors that may introduce bias) and of studies with sufficiently large numbers of the health outcome of interest. For example, for elucidating our understanding of LDL-C lowering therapies, many common variants in *HMGCR* are needed to mimic the biological mechanism by which statins exert their effects, as individual variants are associated with only small life-long differences in LDL-C levels. By contrast, single functional variants in the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) can be used to mimic pharmacological PCSK9-inihibition as they have large (about 10-fold greater) life-long effects on LDL-C levels.[4–7]

The present study assesses the association of *PCSK9* genetic variants with risk among more than 10 000 well-characterized IS cases, and compares its strength with the association for CHD risk.

Methods

Selection of *PCSK9* genetic variants

PCSK9 variants were selected based on known variation in European ancestry groups, functional relevance and previously published associations. The primary focus was on the loss-of-function missense rs11591147 (R46L) polymorphism because, although relatively uncommon, it has a large effect on PCSK9 and LDL-C levels, and has been found to be strongly associated with CHD.[4,5] In addition, three missense variants (rs562556 [V474I], rs505151[E670G], and rs11583680 [A53V]) and two non-coding variants (rs11206510 and rs2479409) were examined because, although associated with smaller effects on LDL-C and CHD, they are relatively common.[4,8–11]

Study populations

Data were taken from three genome-wide meta-analyses: the Global Lipids Genetics Consortium (GLGC), CARDIoGRAMPlusC4D, and METASTROKE.[10,12,13] The GLGC meta-analysis provides genome-wide associations of these *PCSK9* variants with LDL-C (and other lipids) in up to 188 577 participants, ranging from 77 417 participants for rs11591147 (R46L) to 172 970 participants for rs2479409.[10] CARDIoGRAMPlusC4D provides genome-wide associations (from a 1000 Genomes imputation) with CHD risk in up to 60 801 CHD cases and 123 504 controls from 48 studies among individuals of predominantly European ancestry, with data available for rs11591147 [R46L] in 37 748 CHD cases and 97 202 controls from 31 studies.[12]

The METASTROKE collaboration provides genome-wide associations (from a 1000 Genomes imputation) with IS risk for all of these *PCSK9* variants in 10 307 IS cases and 19 326 controls of European ancestry from 12 studies.[13] The majority of IS cases were recruited through acute stroke services or population studies, and were confirmed by brain imaging. Subtypes of IS (i.e. 1859 cardioembolic, 1817 large artery, and 1349 small vessel cases) were available in nine of the studies based on Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classifications using clinical, imaging and risk factor data.[14] Details of each contributing study are provided in the Supplementary materials, and additional information about the data collection and genetic data quality control procedures is reported elsewhere.[13]

Statistical analyses

Per-allele effects of the selected *PCSK9* variants on LDL-C levels were extracted from the GLGC meta-analysis and converted from the published standard deviation units to mmol/L (based on 1 standard deviation being equivalent to 1.0083 mmol/L, as estimated by a pooled standard deviation from available studies contributing to GLGC).[10] Per-allele log-odds effects of the variants on CHD risk were taken from the CARDIoGRAMPlusC4D genome-wide meta-analysis.[12] In the METASTROKE meta-analysis, per-allele log-odds effects for each *PCSK9* variant were estimated in a fixed-effects meta-analysis for total IS and each IS subtype (large artery, cardioembolic, and small vessel disease).[13] Estimates for disease phenotypes are given with respect to the LDL-C lowering allele. To account for multiple testing, an adjusted significance level of $P < 0.008$ was predefined for resulting associations. The effects of independent genetic variants were combined in a *PCSK9* genetic risk score using an inverse-variance approach, weighted by LDL-C estimates taken from the GLGC. Cochran's Q statistic was used to test for heterogeneity. Analyses were performed in SAS v9.

Results

Impact of the rs11591147 (R46L) variant

The low frequency (1.5%) rs11591147 (R46L) loss-of-function variant was associated with a 0.5 mmol/L [95% confidence interval (CI) 0.47–0.54, $P = 9 \times 10^{-143}$] lower LDL-C level per T allele. The LDL-lowering allele was associated with a 23% lower risk of CHD [odds ratio (OR) 0.77, 95% CI: 0.69–0.87, $P = 7 \times 10^{-6}$, *Figure 1*]. By contrast, the variant was not associated with IS risk (OR: 1.04, 95% CI: 0.84–1.28, $P = 0.74$, *Figure 1*), and there was no significant heterogeneity of the effect on IS between the contributing studies ($P = 0.26$). Overall, the association of rs11591147 (R46L) with IS risk was significantly different to that with CHD risk (P for heterogeneity $= 0.02$). These analyses had about 80% power at $P < 0.008$ (i.e. making allowance for multiple comparisons) to detect an effect for IS that was as large as that for CHD (although a smaller effect on IS cannot be excluded).

Impact of additional *PCSK9* variants

The five additional *PCSK9* variants that were examined ranged in frequency from 14.1% to 96.5% for the LDL-lowering allele. Each of the additional *PCSK9* variants was significantly associated with LDL-C levels, with effects ranging from 0.09 mmol/L lower LDL-C per allele (95% CI: 0.07–0.11, $P = 4 \times 10^{-17}$) for rs505151 to 0.03 mmol/L lower LDL-C per allele (95% CI: 0.02–0.05, $P = 1 \times 10^{-8}$) for rs11583680 (*Table 1*). Although these variants appeared to be independent of rs11591147 (R46L) based on measures of r^2, the D' measure of genetic departure from independence was >0.6 for all except rs505151 (see Supplementary material online, *Table S1*). Consequently, the effects of each variant on risk were examined separately and a weighted genetic risk score was constructed including only the two independent variants (rs11591147 and rs505151).

Figure 2 illustrates the weaker effect of each of these variants on IS risk than on CHD risk plotted against their effect on

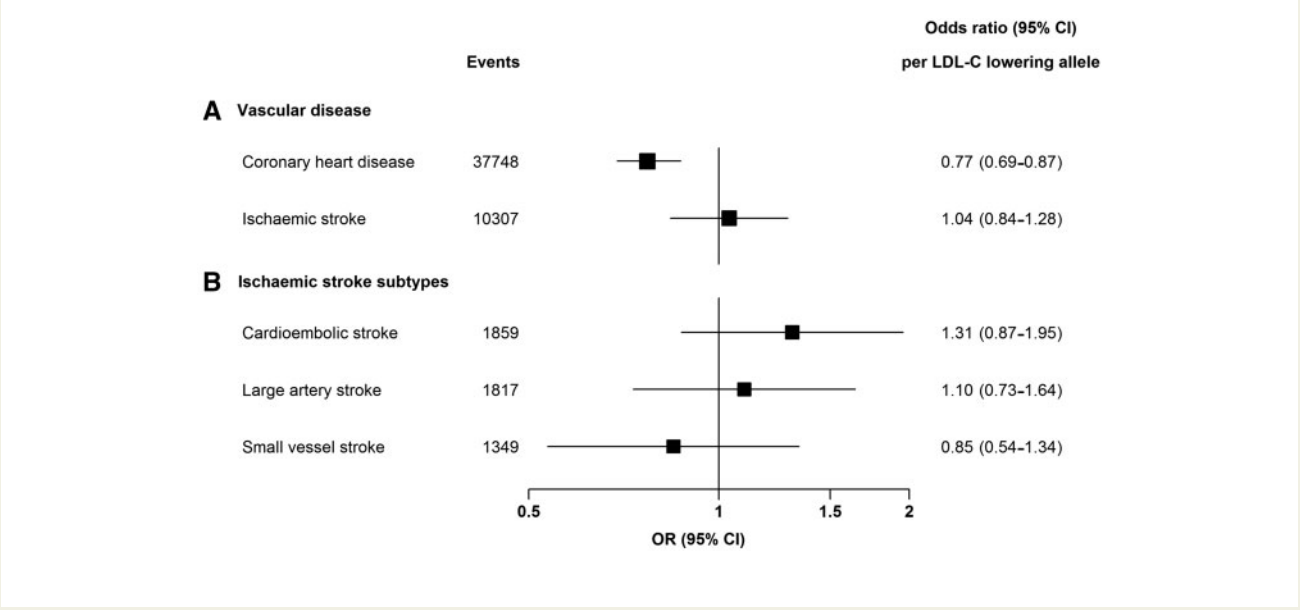


Figure 1 Associations of the *PCSK9* rs11591147 (R46L) variant (per LDL-C lowering allele) with risk of (*A*) coronary heart disease and ischaemic stroke and with (*B*) ischaemic stroke subtypes. Odds ratios (OR) and 95% confidence intervals (CI) are provided per T allele for each outcome. LDL-C, low-density lipoprotein cholesterol.

Table 1 Effects of *PCSK9* variants on low-density lipoprotein cholesterol, coronary heart disease and ischaemic stroke

SNP	Effect/other allele	Effect allele frequency	LDL-C reduction		CHD		Ischaemic stroke		*P* Het
			mmol/L (95% CI)	*P*	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	(CHD vs. IS)
rs11591147	T/G	1.5%	0.50 (0.47–0.54)	9×10^{-143}	0.77 (0.69–0.87)	7×10^{-6}	1.04 (0.84–1.28)	0.74	0.02
rs505151	A/G	96.5%	0.09 (0.07–0.11)	4×10^{-17}	0.96 (0.92–1.00)	0.07	1.00 (0.90–1.11)	0.98	0.50
rs11206510[a]	C/T	18.8%	0.08 (0.07–0.09)	2×10^{-53}	0.93 (0.90–0.95)	2×10^{-8}	1.01 (0.96–1.06)	0.84	0.01
rs2479409[a]	A/G	65.5%	0.06 (0.06–0.07)	3×10^{-50}	0.97 (0.95–0.99)	0.01	1.00 (0.95–1.04)	0.86	0.31
rs562556[a]	G/A	18.3%	0.06 (0.05–0.08)	6×10^{-21}	1.00 (0.98–1.03)	>0.99	1.06 (1.01–1.11)	0.03	0.05
rs11583680[a]	T/C	14.1%	0.03 (0.02–0.05)	1×10^{-8}	0.97 (0.94–1.00)	0.03	1.00 (0.94–1.06)	0.89	0.40
Genetic score (per 1 mmol/L lower LDL-C)					0.60 (0.49–0.74)	1×10^{-6}	1.07 (0.71–1.60)	0.76	0.01

LDL-C effect estimates are based on up to 172 970 individuals from the Global Lipids Consortium, CHD effect estimates are based on up to 60 801 CHD cases and 123 504 controls from the CARDIoGRAMplusC4D Consortium, and IS effect estimates are based on 10 307 IS cases and 19 326 controls from the METASTROKE Consortium. The genetic score is based on independent variants rs11591147 and rs505151. Estimates are given per effect allele unless otherwise stated.

LDL-C, low-density lipoprotein cholesterol; CHD, coronary heart disease; IS, ischaemic stroke; OR, odds ratio; CI, confidence interval; P Het, P for heterogeneity (1 degree of freedom) between CHD and IS.

[a]Variant is in linkage disequilibrium with rs11591147 (D'>0.6), for further information see Supplementary material online, *Table S1*.

LDL-C level. Their effects on CHD were generally consistent with their effects on LDL-C, although there was some evidence of heterogeneity of the CHD effect sizes when they were scaled to the same LDL-C difference ($P = 0.02$). Consistent with the result for the rs11591147 (R46L) variant, there were no apparent associations of any of the other *PCSK9* variants with IS risk, and no significant heterogeneity between the effects of all six variants on IS risk when scaled to the same LDL-C difference ($P = 0.56$).

There were also no significant associations of rs11591147 (R46L) or any of the other *PCSK9* variants with cardioembolic, large artery or small vessel disease stroke (*Figure 1* and *Table 2*).

The *PCSK9* genetic risk score was associated with a 40% lower risk of CHD (OR: 0.60, 95% CI: 0.49–0.74, $P = 1 \times 10^{-6}$) but a non-significant effect on IS (OR: 1.07, 95% CI: 0.71–1.60, $P = 0.76$) per 1 mmol/L lower LDL-C, suggesting significant heterogeneity between the effects of *PCSK9* on CHD and on IS risk (*P* for heterogeneity = 0.01).

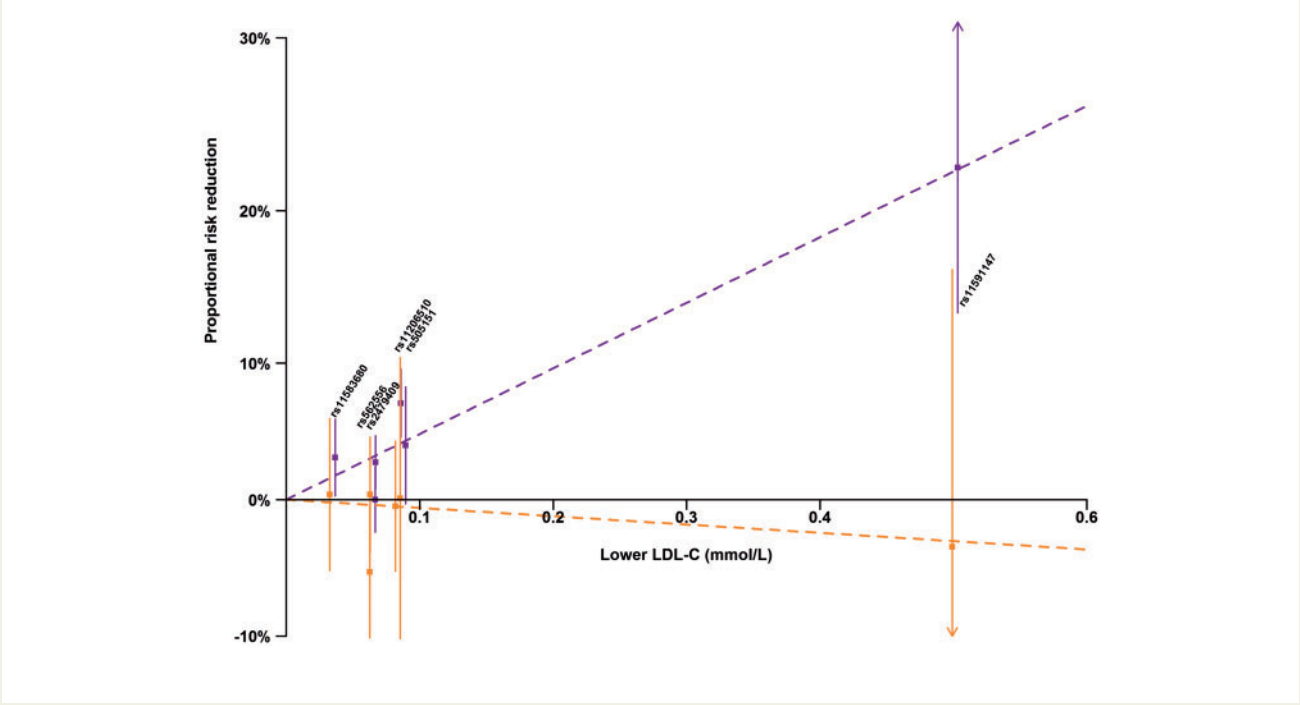


Figure 2 Proportional risk reduction of *PCSK9* variants on coronary heart disease and ischaemic stroke (per LDL-lowering allele) vs. absolute LDL-lowering effects. Effects on coronary heart disease (CHD) risk are shown in purple and effects on ischaemic stroke (IS) risk are shown in gold with dashed lines representing the estimated effects on risk based on the genetic risk score. Plotted points for CHD and IS are equally offset from the estimated effect on LDL-C to avoid overlap. LDL-C, low-density lipoprotein cholesterol.

Discussion

The present study is the first large-scale assessment of associations between *PCSK9* genetic variants and the risk of IS. *PCSK9* variants that confer life-long lower PCSK9 and LDL-C levels, and lower CHD risk, were not associated with risk of IS or IS subtypes. By contrast, in the recently reported FOURIER randomized controlled trial, PCSK9-inhibitor therapy that lowered LDL-C levels by about 1.5 mmol/L reduced the rates of both myocardial infarction and IS by about one-quarter over 2 years.[15] In the SPIRE outcomes trials of PCSK9-inhibitor therapy which were prematurely terminated (and so involved smaller numbers of events), the rate of non-fatal stroke was also reduced by at least as much as for non-fatal myocardial infarction.[16] These findings raise interesting questions about the cause of these apparent differences, as well as about the use of Mendelian randomization to predict the effects of novel therapeutic interventions.[17]

Impact of *PCSK9* on cardiovascular risk factors

Mendelian randomization studies that assess the causal relevance of a risk factor for an outcome can avoid the reverse causation and confounding common in observational studies.[18] However, pleiotropy in which a single locus directly influences multiple phenotypes can complicate their interpretation.[19] The *PCSK9* genetic variants that were examined in the present study have previously been shown to have potential ($P < 0.05$) pleiotropic associations with non-LDL-C cardiovascular risk factors.[20,21] For example, *PCSK9* variants associated with lower LDL-C have also been found to be associated with lower triglyceride and Lp(a) levels, as well as with differences in lipid metabolites and higher levels of HDL-C, fasting glucose, bodyweight, and rates of diabetes.[7,10,21–28] However, many of these non-LDL-C effects of *PCSK9* variants are shared by therapeutic PCSK9-inhibition.[29–32] Although the present study is not a direct test of the effects of LDL-C on IS risk (but, instead, is a test of PCSK9 levels on IS risk), the magnitude of association of rs11591147 (R46L) with CHD risk is broadly consistent with that predicted from Mendelian randomization studies of LDL-C and CHD.[33,34] Hence, the CHD results are consistent with the effects of *PCSK9* being mediated chiefly through changes in LDL-C, with the combined impact of any other effects being only small or neutral.

Large-scale Mendelian randomization studies are needed that directly assess the relevance of specific, as well as varied, biological pathways that lower LDL-C in order to establish the broader causal relevance of life-long variation in LDL-C levels for IS risk. For example, variants in *HMGCR* could be used to examine the impact of the particular mechanism that is targeted by statin therapy, and its consequences on risk of IS and IS subtypes. However, to investigate the wider causal relevance of LDL-C levels (in contrast to PCSK9, or any other, drug target specifically), a genetic risk score which combines variants across the genome that reflect varied pathways (but are specifically associated with LDL-C levels) would be appropriate; such a score could also explain a greater proportion of the variance

Table 2 Effects of *PCSK9* variants on ischaemic stroke subtypes

SNP	Effect/other allele	Effect allele frequency	Cardioembolic stroke		Large artery stroke		Small vessel disease	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs11591147	T/G	1.5%	1.31 (0.87–1.95)	0.20	1.10 (0.73–1.64)	0.66	0.85 (0.54–1.34)	0.48
rs505151	A/G	96.5%	0.96 (0.78–1.16)	0.65	0.99 (0.80–1.21)	0.89	0.89 (0.71–1.11)	0.30
rs11206510[a]	C/T	18.8%	0.97 (0.88–1.06)	0.48	1.00 (0.91–1.10)	0.98	0.95 (0.85–1.05)	0.32
rs2479409[a]	A/G	65.5%	1.00 (0.92–1.09)	0.98	0.98 (0.90–1.07)	0.73	0.97 (0.89–1.07)	0.59
rs562556[a]	G/A	18.3%	1.06 (0.96–1.17)	0.24	1.12 (1.02–1.24)	0.02	0.99 (0.89–1.11)	0.88
rs11583680[a]	T/C	14.1%	0.95 (0.85–1.07)	0.42	0.97 (0.87–1.09)	0.64	0.94 (0.83–1.07)	0.35
Genetic score (per 1 mmol/L lower LDL-C)			1.51 (0.71–3.23)	0.28	1.16 (0.54–2.48)	0.71	0.64 (0.27–1.52)	0.31

Cardioembolic stroke effect estimates are based on 1859 cases, large artery stroke effect estimates are based on 1817 cases, and small vessel disease effect estimates are based on 1349 cases. The genetic score is based on independent variants rs11591147 and rs505151. Estimates are given per effect allele unless otherwise stated.
OR, odds ratio; CI, confidence interval.
[a]Variant is in linkage disequilibrium with rs11591147 (D' >0.6), for further information see Supplementary material online, *Table S1*.

in LDL-C levels than any single variant and thereby provide greater statistical power.[35] Assessment of the effects of a genetic score in studies involving sufficiently large numbers of disease cases is needed to determine reliably the effects of life-long differences in LDL-C on IS and IS subtypes.

Impact of *PCSK9* on coronary heart disease and ischaemic stroke

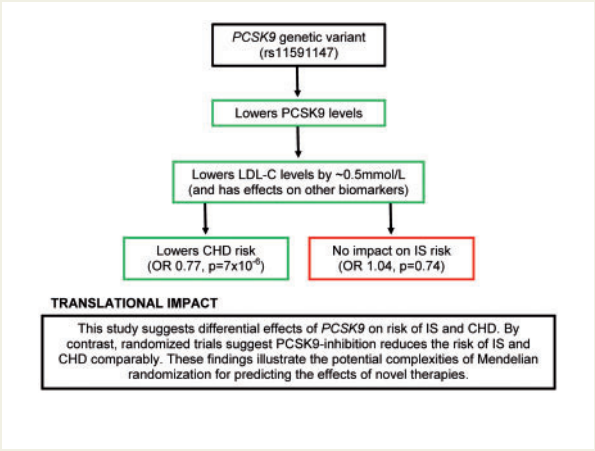
The observed effects of *PCSK9* variants on CHD in the present study are broadly consistent with those reported previously.[4,6,33,34] For example, Ference *et al.*[6] reported a 28% (95% CI: 16% –39%) lower risk per rs11591147 T allele (albeit the CHD cases overlapped somewhat with those in the present study), and Benn *et al.*[4] found a 30% (95% CI: 14–42%) lower risk of CHD in carriers of the LDL-lowering T allele. However, there did not appear to be an association of rs11591147 with stroke risk in the initial studies of *PCSK9* among individuals of African American or European ancestry, although the number of strokes observed was small.[4,5] In other candidate-gene studies, the observed associations of *PCSK9* variants with IS risk have been inconsistent.[36–39] Studies of other functional *PCSK9* variants that produce large effects on PCSK9 and LDL-C levels (e.g. mutations in Y142X or C679X associated with life-long differences in LDL-C levels of about 1 mmol/L) have not detected associations with stroke.[5,40] However, such loss-of-function mutations are rare (about 2% frequency in individuals of African ancestry and far lower frequency in individuals of European ancestry) and the existing studies have involved only about 200 stroke cases.

In a recently reported study involving 3675 cases of stroke of any aetiology, the association of a *PCSK9* genetic risk score (that excluded the rs11591147 variant, which has a considerably larger effect on LDL-C levels than the variants considered) with the risk of stroke was also weaker (OR: 0.96, 95% CI: 0.90–1.01) than was the association with non-fatal myocardial infarction (OR: 0.89, 95% CI: 0.85–0.94), when comparing participants with a *PCSK9* score above and below the median.[7] That observation is less robust than the present results since it was based on about one-third as many strokes, and was not restricted to strokes of ischaemic origin. Stroke research has often been complicated by diagnostic limitations and phenotypic heterogeneity (i.e. combining strokes of ischaemic and haemorrhagic aetiology), as well as by an inability to distinguish IS subtypes. However, in the present study, the majority of IS cases were confirmed and subtyped based on brain imaging and/or a specialist neurologist examination, reducing the potential impact of misclassification. Nonetheless, much larger studies are needed for reliable assessment of genetic effects on aetiologically distinct IS subtypes and to assess any heterogeneity between them, which may elucidate our understanding of the relationship between PCSK9, LDL-C, and IS further.

Differences in the impact of *PCSK9* on the risks of IS and CHD in the present study may represent biological differences, such as relative differences in the contribution of lipids, as well as phenotypic and design artefacts. Exploration of canonical pathways has suggested that genetic determinants of CHD are linked to lipid metabolism pathways whereas, by contrast, those for IS are linked to natural killer cell signalling rather than to lipid pathways.[9,13] IS also involves phenotypic heterogeneity, with different biological drivers for cardioembolic, large artery and small vessel disease, by contrast to the more homogenous CHD phenotype. Genetic associations may be stronger for disease cases that occur at a younger age which, in principle, could be a confounding factor in the present comparison between *PCSK9* associations for CHD and IS derived from different studies. However, the age range for CHD and IS cases in the studies contributing to these analyses was similar, so the impact of age-related confounding is likely to be limited. Studies involving larger numbers of CHD and IS cases within the same study may well help to elucidate the role of such factors in Mendelian randomization studies.

Mendelian randomization vs. randomized trials

ESC/EAS guidelines recommend consideration of PCSK9-inhibitor therapy in very high-risk patients with persistently high LDL-C levels despite treatment with maximal tolerated statin therapy plus ezetimibe.[41] Based on the present study, PCSK9-inhibitor therapy would have been predicted to have a significantly weaker, if any, effect



Take home figure Overview of the impact of *PCSK9* variants on CHD and IS.

on risk of IS than on risk of CHD. Recent randomized trials have shown comparable effects of PCSK9-inhibition on IS and myocardial infarction, although the lower confidence limits are also consistent with a weaker effect on IS than on myocardial infarction (8% vs. 18% risk reduction, respectively).[15] The FOURIER and SPIRE trials recruited populations at high risk of atherosclerotic events, with all participants in FOURIER having clinically evident atherosclerotic cardiovascular disease (including 80% with a history of myocardial infarction) and almost 85% of participants in SPIRE-1 and SPIRE-2 having had a previous cardiovascular event (and the remaining 15% being a high risk primary prevention cohort).[15,16] By contrast, the risk factor and comorbidity profiles of individuals included in the METASTROKE studies represent a population that is less enriched for atherosclerotic disease. Consequently, by comparison with the trials, a larger proportion of IS events in METASTROKE may have been driven by risk factors that are not amenable to lipid modification (such as hypertension and atrial fibrillation), resulting in attenuation of the genetic associations. Further large studies are needed with sufficient statistical power to consider the impact of population characteristics (such as risk of disease) on the results of Mendelian randomization studies.

Conclusion

PCSK9 genetic variants that produce life-long lower levels of LDL-C, and that are associated with lower risk of CHD, appear to have a significantly weaker (if any) effect on the risk of IS. By contrast, PCSK9-inhibitor therapy (as with statin therapy and ezetimibe) has been shown to reduce the risk of CHD and stroke comparably. These findings illustrate potential limitations with the use of Mendelian randomization to predict the effects of novel therapeutic interventions on different health outcomes.[17]

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Summary results for LDL-C have been contributed by the Global Lipids Genetics Consortium, downloaded at http://csg.sph.umich.edu//abecasis/public/lipids2013/. Summary results for coronary heart disease have been contributed by the CARDIoGRAMplusC4D investigators, downloaded at www.CARDIOGRAMPLUSC4D.ORG. Results for ischaemic stroke have been contributed by the International Stroke Genetics Consortium METASTROKE collaboration, with acknowledgements to each of the contributing studies provided in the Supplementary material.

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Conflict of interest: none declared.

Translational perspectives

Mendelian randomization may provide an assessment of the effects of modifying specific biological pathways that is less prone to confounding. In the present study, *PCSK9* genetic variants associated with life-long lower levels of PCSK9 and low-density lipoprotein cholesterol (LDL-C) were associated with lower risk of coronary heart disease (CHD) but not of ischaemic stroke (IS). By contrast, in randomized trials, PCSK9-inhibitor therapy (as found with statins and ezetimibe) produces similar proportional reductions in the risks of CHD and IS. Even so, both the Mendelian randomization and clinical trial evidence are statistically consistent with there being a weaker effect of PCSK9 on IS risk than on CHD risk. These findings indicate that there may be complexities in the relationship between PCSK9, LDL-C, and IS, as well as with the use of Mendelian randomization to predict the effects of interventions, that require further study.

References

1. The Emerging Risk Factors Collaboration. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;**302**:1993–2000.
2. The Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010;**376**:1670–1681.
3. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med* 2015;**372**:2387–2397.
4. Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjaerg-Hansen A. *PCSK9* R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *J Am Coll Cardiol* 2010;**55**:2833–2842.
5. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;**354**:1264–1272.
6. Ference BA, Majeed F, Penumetcha R, Flack JM, Brook RD. Effect of naturally random allocation to lower low-density lipoprotein cholesterol on the risk of coronary heart disease mediated by polymorphisms in *NPC1L1*, *HMGCR*, or both: a 2 x 2 factorial Mendelian randomization study. *J Am Coll Cardiol* 2015;**65**:1552–1561.
7. Ference BA, Robinson J, Brook RD, Catapano AL, Chapman J, Neff DR, Voros S, Guigliano RP, Davey Smith G, Fazio S, Sabatine MS. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med* 2016;**375**:2144–2153.
8. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, Cohen JC, Hobbs HH. A spectrum of *PCSK9* alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am J Hum Genet* 2006;**78**:410–422.
9. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, Konig IR, Cazier JB, Johansson A, Hall AS, Lee JY, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lyytikainen LP, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R, Doney AS, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Muller-Nurasyid M, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schafer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot CE, Wagner PJ, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D, Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A, Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrieres J, Gauguier D, Go AS, Goodall AH, Gudnason V, Hazen SL, Holm H, Iribarren C, Jang Y, Kahonen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W, Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouwehand WH, Parish S, Park JE, Pedersen NL, Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K, Tregouet DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C, Sandhu MS, Pastinen T, Syvanen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimaki T, Metspalu A, Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehm BO, O'donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ., The CARDIoGRAMplusC4D Consortium Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013;**45**:25–33.
10. The Global Lipids Genetics Consortium. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013;**45**:1274–1283.
11. Ding K, Kullo IJ. Molecular population genetics of *PCSK9*: a signature of recent positive selection. *Pharmacogenet Genomics* 2008;**18**:169–179.
12. The CARDIoGRAMplusC4D Consortium. A comprehensive 1000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015;**47**:1121–1130.
13. Malik R, Traylor M, Pulit SL, Bevan S, Hopewell JC, Holliday EG, Zhao W, Abrantes P, Amouyel P, Attia JR, Battey TW, Berger K, Boncoraglio GB, Chauhan G, Cheng YC, Chen WM, Clarke R, Cotlarciuc I, Debette S, Falcone GJ, Ferro JM, Gamble DM, Ilinca A, Kittner SJ, Kourkoulis CE, Lemmens R, Levi CR, Lichtner P, Lindgren A, Liu J, Meschia JF, Mitchell BD, Oliveira SA, Pera J, Reiner AP, Rothwell PM, Sharma P, Slowik A, Sudlow CL, Tatlisumak T, Thijs V, Vicente AM, Woo D, Seshadri S, Saleheen D, Rosand J, Markus HS, Worrall BB, Dichgans M. Low-frequency and common genetic variation in ischemic stroke: the METASTROKE collaboration. *Neurology* 2016;**86**:1217–1226.
14. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993;**24**:35–41.
15. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med* 2017;**376**:1713–1722.
16. Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F, Flather M, Glynn RJ, Gregoire J, Jukema JW, Karpov Y, Kastelein JJ, Koenig W, Lorenzatti A, Manga P, Masiukiewicz U, Miller M, Mosterd A, Murin J, Nicolau JC, Nissen S, Ponikowski P, Santos RD, Schwartz PF, Soran H, White H, Wright RS, Vrablik M, Yunis C, Shear CL, Tardif JC. Cardiovascular efficacy and safety of Bococizumab in high-risk patients. *N Engl J Med* 2017;**376**:1527–1539.
17. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. *Nat Rev Cardiol* 2017;doi:10.1038/nrcardio.2017.78.
18. Davey Smith G, Ebrahim S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ* 2005;**330**:1076–1079.
19. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet* 2014;**23**:R89–R98.
20. PhenoScanner: a database of human genotype-phenotype associations. www.phenoscanner.medschl.cam.ac.uk (11 November 2016).
21. Staley JR, Blackshaw J, Kamat MA, Ellis S, Surendran P, Sun BB, Paul DS, Freitag D, Burgess S, Danesh J, Young R, Butterworth AS. PhenoScanner: a database of human genotype-phenotype associations. *Bioinformatics* 2016;**32**:3207–3209.
22. Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Malarstig A, Ordovas JM, Ripatti S, Parker AN, Miletich JP, Ridker PM. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. *PLoS Genet* 2009;**5**:e1000730.
23. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker PI, O'donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL, Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009;**41**:56–65.
24. Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikainen LP, Kangas AJ, Soininen P, Wurtz P, Silander K, Dick DM, Rose RJ, Savolainen MJ, Viikari J, Kahonen M, Lehtimaki T, Pietilainen KH, Inouye M, McCarthy MI, Jula A, Eriksson J, Raitakari OT, Salomaa V, Kaprio J, Jarvelin MR, Peltonen L, Perola M, Freimer NB, Ala-Korpela M, Palotie A, Ripatti S. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet* 2012;**44**:269–276.
25. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Magi R, Strawbridge RJ, Rehnberg E, Gustafsson S, Kanoni S, Rasmussen-Torvik LJ, Yengo L, Lecoeur C, Shungin D, Sanna S, Sidore C, Johnson PC, Jukema JW, Johnson T, Mahajan A, Verweij N, Thorleifsson G, Hottenga JJ, Shah S, Smith AV, Sennblad B, Gieger C, Salo P, Perola M, Timpson NJ, Evans DM, Pourcain BS, Wu Y, Andrews JS, Hui J, Bielak LF, Zhao W, Horikoshi M, Navarro P, Isaacs A, O'connell JR, Stirrups K, Vitart V, Hayward C, Esko T, Mihailov E, Fraser RM, Fall T, Voight BF, Raychaudhuri S, Chen H, Lindgren CM, Morris AP, Rayner NW, Robertson N, Rybin D, Liu CT, Beckmann JS, Willems SM, Chines PS, Jackson AU, Kang HM, Stringham HM, Song K, Tanaka T, Peden JF, Goel A, Hicks AA, An P, Muller-Nurasyid M, Franco-Cereceda A, Folkersen L, Marullo L, Jansen H, Oldehinkel AJ, Bruinenberg M, Pankow JS, North KE, Forouhi NG, Loos RJ, Edkins S, Varga TV, Hallmans G, Oksa H, Antonella M, Nagaraja R, Trompet S, Ford I, Bakker SJ, Kong A, Kumari M, Gigante B, Herder C, Munroe PB, Caulfield M, Antti J, Mangino M, Small K, Miljkovic I, Liu Y, Atalay M, Kiess W, James AL, Rivadeneira F, Uitterlinden AG, Palmer CN, Doney AS, Willemsen G, Smit JH, Campbell S, Polasek O, Bonnycastle LL, Hercberg S, Dimitriou M, Bolton JL, Fowkes GR, Kovacs P, Lindstrom J, Zemunik T, Bandinelli S, Wild SH, Basart HV, Rathmann W, Grallert H, Maerz W, Kleber ME, Boehm BO, Peters A, Pramstaller PP, Province MA, Borecki IB, Hastie ND, Rudan I, Campbell H, Watkins H, Farrall M, Stumvoll M, Ferrucci L, Waterworth DM, Bergman RN, Collins FS, Tuomilehto J, Watanabe RM, de Geus EJ, Penninx BW, Hofman A, Oostra BA, Psaty BM, Vollenweider P, Wilson JF, Wright AF, Hovingh GK, Metspalu A, Uusitupa M, Magnusson PK, Kyvik KO, Kaprio J, Price JF, Dedoussis GV, Deloukas P, Meneton P, Lind L, Boehnke M, Shuldiner AR, van Duijn CM, Morris AD, Toenjes A, Peyser PA, Beilby JP, Korner A, Kuusisto J, Laakso M, Bornstein SR, Schwarz PE, Lakka TA, Rauramaa R, Adair LS, Smith GD, Spector

TD, Illig T, de Faire U, Hamsten A, Gudnason V, Kivimaki M, Hingorani A, Keinanen-Kiukaanniemi SM, Saaristo TE, Boomsma DI, Stefansson K, van der Harst P, Dupuis J, Pedersen NL, Sattar N, Harris TB, Cucca F, Ripatti S, Salomaa V, Mohlke KL, Balkau B, Froguel P, Pouta A, Jarvelin MR, Wareham NJ, Bouatia-Naji N, McCarthy MI, Franks PW, Meigs JB, Teslovich TM, Florez JC, Langenberg C, Ingelsson E, Prokopenko I, Barroso I. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet* 2012;**44**:991–1005.

26. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Magi R, Strawbridge RJ, Pers TH, Fischer K, Justice AE, Workalemahu T, Wu JM, Buchkovich ML, Heard-Costa NL, Roman TS, Drong AW, Song C, Gustafsson S, Day FR, Esko T, Fall T, Kutalik Z, Luan J, Randall JC, Scherag A, Vedantam S, Wood AR, Chen J, Fehrmann R, Karjalainen J, Kahali B, Liu CT, Schmidt EM, Absher D, Amin N, Anderson D, Beekman M, Bragg-Gresham JL, Buyske S, Demirkan A, Ehret GB, Feitosa MF, Goel A, Jackson AU, Johnson T, Kleber ME, Kristiansson K, Mangino M, Mateo Leach I, Medina-Gomez C, Palmer CD, Pasko D, Pechlivanis S, Peters MJ, Prokopenko I, Stancakova A, Ju Sung Y, Tanaka T, Teumer A, Van Vliet-Ostaptchouk JV, Yengo L, Zhang W, Albrecht E, Arnlov J, Arscott GM, Bandinelli S, Barrett A, Bellis C, Bennett AJ, Berne C, Bluher M, Bohringer S, Bonnet F, Bottcher Y, Bruinenberg M, Carba DB, Caspersen IH, Clarke R, Daw EW, Deelen J, Deelman E, Delgado G, Doney AS, Eklund N, Erdos MR, Estrada K, Eury E, Friedrich N, Garcia ME, Giedraitis V, Gigante B, Go AS, Golay A, Grallert H, Grammer TB, Grassler J, Grewal J, Groves CJ, Haller T, Hallmans G, Hartman CA, Hassinen M, Hayward C, Heikkila K, Herzig KH, Helmer Q, Hillege HL, Holmen O, Hunt SC, Isaacs A, Ittermann T, James AL, Johansson I, Juliusdottir T, Kalafati IP, Kinnunen L, Koenig W, Kooner IK, Kratzer W, Lamina C, Leander K, Lee NR, Lichtner P, Lind L, Lindstrom J, Lobbens S, Lorentzon M, Mach F, Magnusson PK, Mahajan A, McArdle WL, Menni C, Merger S, Mihailov E, Milani L, Mills R, Moayyeri A, Monda KL, Mooijaart SP, Muhleisen TW, Mulas A, Muller G, Muller-Nurasyid M, Nagaraja R, Nalls MA, Narisu N, Glorioso N, Nolte IM, Olden M, Rayner NW, Renstrom F, Ried JS, Robertson NR, Rose LM, Sanna S, Scharnagl H, Scholtens S, Sennblad B, Seufferlein T, Sitlani CM, Vernon Smith A, Stirrups K, Stringham HM, Sundstrom J, Swertz MA, Swift AJ, Syvanen AC, Tayo BO, Thorand B, Thorleifsson G, Tomaschitz A, Troffa C, van Oort FV, Verweij N, Vonk JM, Waite LL, Wennauer R, Wilsgaard T, Wojczynski MK, Wong A, Zhang Q, Hua Zhao J, Brennan EP, Choi M, Eriksson P, Folkersen L, Franco-Cereceda A, Gharavi AG, Hedman AK, Hivert MF, Huang J, Kanoni S, Karpe F, Keildson S, Kiryluk K, Liang L, Lifton RP, Ma B, McKnight AJ, McPherson R, Metspalu A, Min JL, Moffatt MF, Montgomery GW, Murabito JM, Nicholson G, Nyholt DR, Olsson C, Perry JR, Reinmaa E, Salem RM, Sandholm N, Schadt EE, Scott RA, Stolk L, Vallejo EE, Westra HJ, Zondervan KT, Amouyel P, Arveiler D, Bakker SJ, Beilby J, Bergman RN, Blangero J, Brown MJ, Burnier M, Campbell H, Chakravarti A, Chines PS, Claudi-Boehm S, Collins FS, Crawford DC, Danesh J, de Faire U, de Geus EJ, Dorr M, Erbel R, Eriksson JG, Farrall M, Ferrannini E, Ferrieres J, Forouhi NG, Forrester T, Franco OH, Gansevoort RT, Gieger C, Gudnason V, Haiman CA, Harris TB, Hattersley AT, Heliovaara M, Hicks AA, Hingorani AD, Hoffmann W, Hofman A, Homuth G, Humphries SE, Hypponen E, Illig T, Jarvelin MR, Johansen B, Jousilahti P, Jula AM, Kaprio J, Kee F, Keinanen-Kiukaanniemi SM, Kooner JS, Kooperberg C, Kovacs P, Kraja AT, Kumari M, Kuulasmaa K, Kuusisto J, Lakka TA, Langenberg C, Le Marchand L, Lehtimaki T, Lyssenko V, Mannisto S, Marette A, Matise TC, McKenzie CA, McKnight B, Musk AW, Mohlenkamp S, Morris AD, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Palmer LJ, Penninx BW, Peters A, Pramstaller PP, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ridker PM, Ritchie MD, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schwarz PE, Shuldiner AR, Staessen JA, Steinthorsdottir V, Stolk RP, Strauch K, Tonjes A, Tremblay A, Tremoli E, Vohl MC, Volker U, Vollenweider P, Wilson JF, Witteman JC, Adair LS, Bochud M, Boehm BO, Bornstein SR, Bouchard C, Cauchi S, Caulfield MJ, Chambers JC, Chasman DI, Cooper RS, Dedoussis G, Ferrucci L, Froguel P, Grabe HJ, Hamsten A, Hui J, Hveem K, Jockel KH, Kivimaki M, Kuh D, Laakso M, Liu Y, Marz W, Munroe PB, Njolstad I, Oostra BA, Palmer CN, Pedersen NL, Perola M, Perusse L, Peters U, Power C, Quertermous T, Rauramaa R, Rivadeneira F, Saaristo TE, Saleheen D, Sinisalo J, Slagboom PE, Snieder H, Spector TD, Thorsteinsdottir U, Stumvoll M, Tuomilehto J, Uitterlinden AG, Uusitupa M, van der Harst P, Veronesi G, Walker M, Wareham NJ, Watkins H, Wichmann HE, Abecasis GR, Assimes TL, Berndt SI, Boehnke M, Borecki IB, Deloukas P, Franke L, Frayling TM, Groop LC, Hunter DJ, Kaplan RC, O'connell JR, Qi L, Schlessinger D, Strachan DP, Stefansson K, van Duijn CM, Willer CJ, Visscher PM, Yang J, Hirschhorn JN, Zillikens MC, McCarthy MI, Speliotes EK, North KE, Fox CS, Barroso I, Franks PW, Ingelsson E, Heid IM, Loos RJ, Cupples LA, Morris AP, Lindgren CM, Mohlke KL. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015;**518**:187–196.
27. Suchindran S, Rivedal D, Guyton JR, Milledge T, Gao X, Benjamin A, Rowell J, Ginsburg GS, McCarthy JJ. Genome-wide association study of Lp-PLA(2) activity and mass in the Framingham Heart Study. *PLoS Genet* 2010;**6**:e1000928.
28. Schmidt AF, Swerdlow DI, Holmes MV, Patel RS, Fairhurst-Hunter Z, Lyall DM, Hartwig FP, Horta BL, Hypponen E, Power C, Moldovan M, van Iperen E, Hovingh GK, Demuth I, Norman K, Steinhagen-Thiessen E, Demuth J, Bertram L, Liu T, Coassin S, Willeit J, Kiechl S, Willeit K, Mason D, Wright J, Morris R, Wanamethee G, Whincup P, Ben-Shlomo Y, McLachlan S, Price JF, Kivimaki M, Welch C, Sanchez-Galvez A, Marques-Vidal P, Nicolaides A, Panayiotou AG, Onland-Moret NC, van der Schouw YT, Matullo G, Fiorito G, Guarrera S, Sacerdote C, Wareham NJ, Langenberg C, Scott R, Luan J, Bobak M, Malyutina S, Pajak A, Kubinova R, Tamosiunas A, Pikhart H, Husemoen LL, Grarup N, Pedersen O, Hansen T, Linneberg A, Simonsen KS, Cooper J, Humphries SE, Brilliant M, Kitchner T, Hakonarson H, Carrell DS, McCarty CA, Kirchner HL, Larson EB, Crosslin DR, de Andrade M, Roden DM, Denny JC, Carty C, Hancock S, Attia J, Holliday E, Donnell MO, Yusuf S, Chong M, Pare G, van der Harst P, Said MA, Eppinga RN, Verweij N, Snieder H, Christen T, Mook-Kanamori DO, Gustafsson S, Lind L, Ingelsson E, Pazoki R, Franco O, Hofman A, Uitterlinden A, Dehghan A, Teumer A, Baumeister S, Dorr M, Lerch MM, Volker U, Volzke H, Ward J, Pell JP, Smith DJ, Meade T, Maitland-van der Zee AH, Baranova EV, Young R, Ford I, Campbell A, Padmanabhan S, Bots ML, Grobbee DE, Froguel P, Thuillier D, Balkau B, Bonnefond A, Cariou B, Smart M, Bao Y, Kumari M, Mahajan A, Ridker PM, Chasman DI, Reiner AP, Lange LA, Ritchie MD, Asselbergs FW, Casas JP, Keating BJ, Preiss D, Hingorani AD, Sattar N. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2017;**5**:97–105.
29. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein EA. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;**372**:1500–1509.
30. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJ. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;**372**:1489–1499.
31. Navarese EP, Kolodziejczak M, Schulze V, Gurbel PA, Tantry U, Lin Y, Brockmeyer M, Kandzari DE, Kubica JM, D'agostino RB Sr, Kubica J, Volpe M, Agewall S, Kereiakes DJ, Kelm M. Effects of proprotein convertase subtilisin/kexin type 9 antibodies in adults with hypercholesterolemia: a systematic review and meta-analysis. *Ann Intern Med* 2015;**163**:40–51.
32. Li C, Lin L, Zhang W, Zhou L, Wang H, Luo X, Luo H, Cai Y, Zeng C. Efficiency and safety of proprotein convertase subtilisin/kexin 9 monoclonal antibody on hypercholesterolemia: a meta-analysis of 20 randomized controlled trials. *J Am Heart Assoc* 2015;**4**:e001937.
33. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams KA Sr, Flack JM. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J Am Coll Cardiol* 2012;**60**:2631–2639.
34. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, Dale CE, Padmanabhan S, Finan C, Swerdlow DI, Tragante V, van Iperen EP, Sivapalaratnam S, Shah S, Elbers CC, Shah T, Engmann J, Giambartolomei C, White J, Zabaneh D, Sofat R, McLachlan S, Doevendans PA, Balmforth AJ, Hall AS, North KE, Almoguera B, Hoogeveen RC, Cushman M, Fornage M, Patel SR, Redline S, Siscovick DS, Tsai MY, Karczewski KJ, Hofker MH, Verschuren WM, Bots ML, van der Schouw YT, Melander O, Dominiczak AF, Morris R, Ben-Shlomo Y, Price J, Kumari M, Baumert J, Peters A, Thorand B, Koenig W, Gaunt TR, Humphries SE, Clarke R, Watkins H, Farrall M, Wilson JG, Rich SS, de Bakker PI, Lange LA, Davey Smith G, Reiner AP, Talmud PJ, Kivimaki M, Lawlor DA, Dudbridge F, Samani NJ, Keating BJ, Hingorani AD, Casas JP. Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J* 2015;**36**:539–550.
35. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol* 2014;**43**:922–929.
36. Abboud S, Karhunen PJ, Lutjohann D, Goebeler S, Luoto T, Friedrichs S, Lehtimaki T, Pandolfo M, Laaksonen R. Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene is a risk factor of large-vessel atherosclerosis stroke. *PLoS One* 2007;**2**:e1043.
37. Han D, Ma J, Zhang X, Cai J, Li J, Tuerxun T, Hao C, Du L, Lei J. Correlation of *PCSK9* gene polymorphism with cerebral ischemic stroke in Xinjiang Han and Uygur populations. *Med Sci Monit* 2014;**20**:1758–1767.
38. Slimani A, Harira Y, Trabelsi I, Jomaa W, Maatouk F, Hamda KB, Slimane MN. Effect of E670G polymorphism in *PCSK9* gene on the risk and severity of coronary heart disease and ischemic stroke in a Tunisian cohort. *J Mol Neurosci* 2014;**53**:150–157.
39. Zhang L, Song K, Zhu M, Shi J, Zhang H, Xu L, Chen Y. Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) in lipid metabolism, atherosclerosis and ischemic stroke. *Int J Neurosci* 2016;**126**:675–680.
40. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in *PCSK9*. *Nat Genet* 2005;**37**:161–165.
41. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Z, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WM, Vlachopoulos C, Wood DA, Zamorano JL. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J* 2016;**37**:2999–3058.