Atherosclerosis 361 (2022) 41–46

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Genetic proxies for PCSK9 inhibition associate with lipoprotein(a): Effects on coronary artery disease and ischemic stroke

Gian Marco De Marchis [a,b,1,*], Tolga D. Dittrich [a,b,1], Rainer Malik [c], Annaelle V. Zietz [a,b], Lilian F. Kriemler [a,b], Brian A. Ference [d,e], Martin Dichgans [c,f], Marios K. Georgakis [c,f,g,h,**]

[a] Department of Neurology and Stroke Center, University Hospital Basel, Switzerland
[b] Department of Clinical Research, University of Basel, Switzerland
[c] Institute for Stroke and Dementia Research, University Hospital, Ludwig-Maximilians-Universität LMU, Munich, Germany
[d] Centre for Naturally Randomized Trials, University of Cambridge, Cambridge, United Kingdom
[e] MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[f] Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
[g] Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA
[h] Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Boston, MA, USA

ARTICLE INFO

Keywords:
PCSK9 inhibitors
Lipoprotein(a)
Genetics
Stroke
Coronary artery disease

ABSTRACT

Background and aims: Post hoc analyses of clinical trials show that PCSK9 inhibitors might lower lipoprotein(a), but whether this effect contributes to reductions in cardiovascular risk remains unknown. We aimed to assess whether genetically proxied PCSK9 inhibition influences lipoprotein(a) (Lp(a)), and whether any such effect could mediate its effects on coronary artery disease (CAD) and ischemic stroke (IS).

Methods: To explore associations between the genetic proxies for PCSK9 inhibitors and Lp(a) levels, we used UK Biobank data (310,020 individuals). We identified 10 variants in the *PCSK9* gene associated with lower PCSK9 and LDL-C levels as proxies for PCSK9 inhibition. We explored the effects of genetically proxied PCSK9 inhibition on Lp(a) levels, as well as on odds of CAD (60,801 cases, 184,305 controls) and IS (60,341 cases, 454,450 controls) in two-sample Mendelian randomization analyses. In mediation analyses, we assessed the effects of genetically proxied PCSK9 inhibition on CAD and IS mediated through reductions in Lp(a) levels.

Results: Genetically proxied PCSK9 inhibition (1-SD decrement in PCSK9 concentration; corresponding to 20.6 mg/dl decrement in LDL-C levels) was associated with a 4% decrease in log-Lp(a) levels (beta: −0.038, 95%CI: −0.053 to −0.023). We estimated a 0.8% reduction in the odds for CAD (OR: 0.992, 95%CI: 0.989–0.995) and a 0.5% reduction in the odds for atherosclerotic IS (OR: 0.995, 95%CI: 0.992–0.998) due to reductions in Lp(a) levels through genetically proxied PCSK9 inhibition, corresponding to 3.8% and 3.2% of the total effects, respectively.

Conclusions: Genetic proxies for PCSK9 inhibition are associated with lower Lp(a) levels. However, Lp(a) lowering explains only a small proportion of the total effects of genetic proxies for PCSK9 inhibitors on risk of CAD and IS.

1. Introduction

High lipoprotein(a) (Lp(a)) plasma levels are causally associated with atherosclerotic cardiovascular disease [1,2]. However, variance in Lp(a) levels is up to 60% determined by genetic variation in the *LPA* gene on chromosome 6 [3,4], whereas lifestyle modifications, such as diet adjustments only have modest effects on Lp(a) levels [5]. Furthermore, there are so far no approved specific Lp(a) lowering pharmacological agents shown to reduce cardiovascular events. An ongoing phase II study is evaluating olpasiran – a small interfering RNA molecule that

* Corresponding author. Department of Neurology and Stroke Center, University Hospital Basel, Petersgraben 4, 4031, Basel, Switzerland.
** Corresponding author. Institute for Stroke and Dementia Research, University Hospital LMU Munich, Ludwig-Maximilians University (LMU) Munich, Feodor-Lynen Str. 17, D-81377, Munich, Germany.
E-mail addresses: gian.demarchis@usb.ch (G.M. De Marchis), marios.georgakis@med.uni-muenchen.de (M.K. Georgakis).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.atherosclerosis.2022.09.007
Received 6 September 2021; Received in revised form 25 August 2022; Accepted 14 September 2022
Available online 26 September 2022

reduces Lp(a) production in hepatocytes - in individuals with elevated Lp(a) levels (ClinicalTrials.gov Identifier: NCT04270760) [6]. Study completion is expected by the end of 2022. In 2020, a phase II randomized trial showed that the antisense oligonucleotide AKCEA-APO(a)-LRx lowered Lp(a) levels by 80% [7]. The phase III outcome trial HORIZON is ongoing, but results are expected no earlier than 2024 (ClinicalTrials.gov Identifier: NCT04023552) [7].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors were originally developed to reduce cardiovascular events by lowering low-density lipoprotein cholesterol (LDL-C) levels. Alirocumab, in a *post-hoc* analysis of the ODYSSEY OUTCOMES trial, was found to reduce Lp(a) by 25%. Reduction of Lp(a) predicted lower risk of major adverse cardiovascular events (MACE) independent of change from baseline to month 4 in LDL-C [8]. Similarly, Evolocumab, another PCSK9 inhibitor, reduced Lp(a) by 26% in a *post-hoc* analysis of the FOURIER trial. After 12 weeks, lower achieved Lp(a) levels were associated with a lower rate of MACE independently of achieved LDL-C [9]. These findings suggest that PCSK9 inhibition may be a strategy for lowering Lp(a) and thus reduces cardiovascular risk in the absence of specific Lp(a)-lowering approaches. Albeit prespecified, both analyses were *post-hoc*, and a significant effect modification by randomized treatment arm – placebo *vs.* PCSK9 inhibitor – was not shown. Thus, it remains unclear whether any effects of PCSK9 inhibitors on Lp(a) could influence cardiovascular event rates on top of their LDL-C-lowering effects.

Here, we used large-scale genetic data and performed Mendelian randomization (MR) analyses to test this hypothesis. Specifically, we explored whether genetic proxies for PCSK9 inhibition are associated with Lp(a) levels and whether any effect on Lp(a) levels could mediate part of the effects of genetically proxied PCSK9 inhibition on coronary artery disease (CAD) and ischemic stroke (IS).

2. Patients and methods

2.1. Study design and ethical considerations

This Mendelian randomization (MR) study follows the STROBE-MR guidelines [10]. The presented analyses are based on publicly available summary statistics from studies that had obtained ethical approval from their local ethical committees and thus no ethical approval for the current study was required. The data from the UK Biobank are available to all researchers following approval of a research proposal. UK Biobank has approval from the North West Multicentre Research Ethics Committee (MREC) as a Research Tissue Bank (RTB). As data shared from the UK Biobank are deidentified, no additional ethical approval is required. Data for the current project were through the approved application 2532.

An overview of our data sources and our study design is provided in Fig. 1. Briefly, we first identified genetic variants in the *PCSK9* gene that are associated with lower LDL-C levels to be used as proxies (instruments) for PCSK9 inhibition. We then explored whether these genetic proxies for PCSK9 inhibition are associated with circulating Lp(a) levels. Based on the effects of genetically proxied Lp(a) levels on risk of CAD and IS, we finally calculated what proportions of the effects of lifelong genetically proxied PCSK9 inhibition on CAD and IS are mediated through Lp(a) lowering.

2.2. Genetic proxies for PCSK9 inhibition

To identify genetic proxies for PCSK9 inhibition, we used data from a genome-wide association study (GWAS) of the GLGC (Global Lipids Genetics Consortium; http://lipidgenetics.org/) for LDL-C (188,577 individuals) [11]. Given the well-established effect of PCSK9 inhibition on LDL-C levels [12], we used LDL-C levels as a readout for selecting variants in the vicinity of the *PCSK9* gene that mimic PCSK9 inhibition. Specifically, in accordance with previous studies [13,14], we selected genetic variants located within 300 kB of the *PCSK9* gene, which were associated with LDL-C at $p < 5x10^{-8}$. To avoid use of variants in linkage disequilibrium, we clumped variants at an $r^2 < 0.10$ and kept the variants with the lowest *p*-value. We identified 12 genetic variants fulfilling these criteria (Supplementary Table 1). To ensure that the selected variants influenced LDL-C levels through effects on *PCSK9* and not through pleiotropic effects on neighboring genes, we restricted the selection of proxies to variants also associated with plasma PCSK9 concentration (protein quantitative trait loci, pQTLs), as derived from a genomic analyses of plasma proteomic measurements from 35,559 Icelanders included in the Icelandic Cancer Project and various genetic programs at deCODE genetics [15].

As we were interested in the effects of PCSK9 inhibition itself and not of PCSK9-mediated LDL-C lowering on Lp(a) levels, to disentangle the effects of PCSK9 inhibition from the downstream effects of LDL-C lowering, we used as weights for our MR analyses the effects of the variants on plasma PCSK9 concentration. In sensitivity analyses, to derive MR estimates of the effects of genetically proxied PCSK9 inhibition that are comparable to effects for pharmacological PCSK9 inhibition

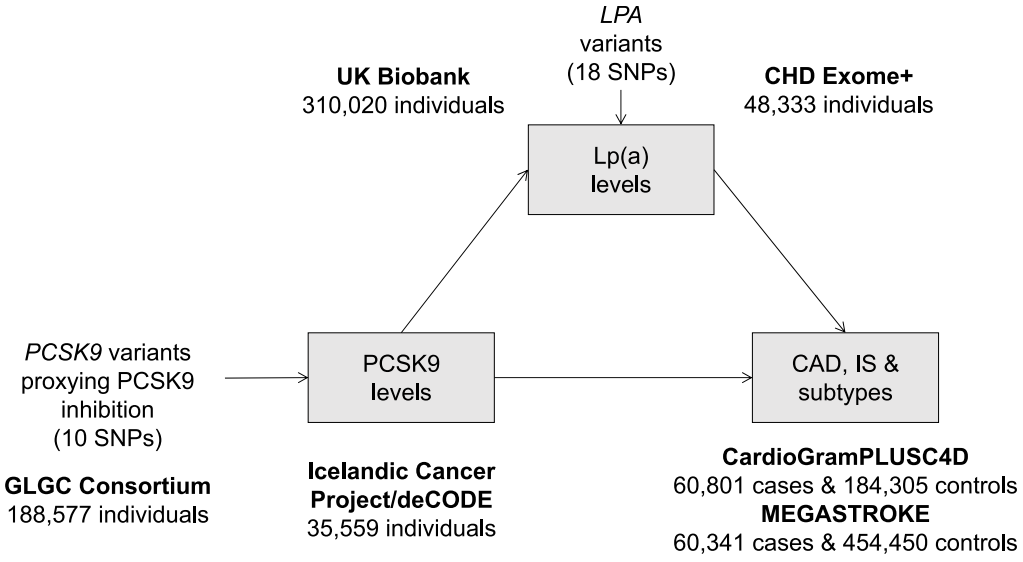

Fig. 1. Study design and data sources used for the current study.

derived from clinical trials, we weighed our variants based on their effects on LDL-C levels.

2.3. Associations with Lp(a) as an outcome

Next, we performed two-sample MR analyses to explore associations between the genetic proxies for PCSK9 inhibitors and Lp(a) levels. For this analysis, we used data from 310,020 individuals from the UK Biobank, a population-based study of individuals aged 40–69 years conducted across multiple centers in the UK [16]. We restricted our analyses to White British individuals (according to self-reported information) who were not under lipid-lowering treatment at the time of Lp(a) measurement and who had available genetic data. We performed the analysis using linear regression adjusting for age, sex, genotyping platform array, assessment center, and the first 20 principal components of the population structure. As a primary approach, we used log-Lp(a) levels to approach a normal distribution. In an alternative approach, we used absolute Lp(a) levels (always measured in mg/dL).

2.4. Genetic proxies for Lp(a) levels

We then leveraged genetic proxies for Lp(a) levels to explore the effects of genetically proxied Lp(a) levels on the risk of cardiovascular outcomes. We used variants within the *LPA* gene region (660 kB window) previously shown to predict Lp(a) levels among 48,333 individuals of the CHD (Coronary Heart Disease) Exome + Consortium [2]. After clumping the initial set of 45 variants at $r^2 < 0.10$, we ended up with 18 variants that were also available in the CardioGramPLUSC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) and MEGASTROKE GWAS (Supplementary Table 2). To avoid differences derived from the different platforms used for measuring Lp(a) levels in the UK Biobank and the CHD Exome + Consortium, we weighed these genetic variants using estimates for log-Lp(a) from the UK Biobank study.

2.5. Associations with cardiovascular outcomes

We finally explored associations of the genetic proxies for PCSK9 inhibitors and for Lp(a) levels with the odds of coronary artery disease, ischemic stroke, and ischemic stroke subtypes. For this purpose, we used publicly available summary statistics from the CardioGramPLUSC4D Consortium for CAD (60,801 cases and 184,305 controls, http://www.cardiogramplusc4d.org/) [17], and from the MEGASTROKE Consortium for ischemic stroke (60,341 cases, 454,450 controls, https://www.megastroke.org/) and its subtypes (large artery atherosclerotic, cardioembolic, small vessel) [18].

2.6. Statistical analysis

For our primary analyses, to explore the effects of the genetic proxies for PCSK9 inhibition on Lp(a) levels, we performed fixed effect inverse-variance weighted (IVW) two-sample MR analyses. We calculated between-variant heterogeneity using the I^2, as a measure of pleiotropy (Cochran's Q-derived $p < 0.05$ was significant) [19]. While the IVW model provides robust and powerful estimates under absence of horizontal pleiotropy, its results can be influenced under presence of pleiotropic effects of the included variants and can thus violate the assumptions of MR analyses [20]. To test the robustness of our results against assumptions of MR analyses, we applied alternative MR methods that are based on different underlying assumptions as sensitivity analyses. Specifically, we applied the weighted median estimator [21], MR-Egger regression [22], and MR-PRESSO [23]. The weighted median estimator allows the use of invalid instruments under the assumption that at least half of the instruments used in the MR analysis are valid [21]. The MR-Egger regression allows for the estimation of an intercept term, which can be used as an indicator of unbalanced directional pleiotropy [22]. 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 [22]. The intercept obtained from MR-Egger regression was used as a measure of directional pleiotropy ($p < 0.05$ indicated statistical significance) [22]. The I^2_{GX} statistic was used as a measure of instrument strength and variability for the MR-Egger analysis [24]. I^2_{GX}<90% indicated high risk of weak instrument bias [24]. MR-PRESSO regresses the variant-outcome estimates against the variant-exposure estimates in order to detect outlier variants [23]. Outliers are detected by sequentially removing all variants from the analyses and comparing the residual sum of squares as a global measure of heterogeneity ($p < 0.05$ for detecting outliers); outliers are then removed and outlier-corrected estimates are provided. MR-PRESSO still relies on the assumption that at least half of the variants are valid instruments [23]. Finally, to exclude the possibility of reverse causation in the association between genetically proxied PCSK9 inhibition and Lp(a) levels, which could influence our MR estimates [25], we performed bidirectional MR exploring the effects of genetically proxied Lp(a) levels on PCSK9 concentration.

To explore whether any effect of the genetic proxies for PCSK9 inhibitors on Lp(a) could explain part of their overall effect on CAD and IS, we then performed two-step mediation MR analyses [26]. We first performed univariable MR weighing these variants on the basis of their associations with log-Lp(a) levels in the UK Biobank and multivariable MR also adjusting for the effects of these variants on PCSK9 concentration to check associations with cardiovascular outcomes. Then, by multiplying the effects of the genetic proxies for PCSK9 inhibition on Lp(a) levels with the multivariable MR association estimates between genetically proxied Lp(a) and the cardiovascular outcomes, we obtained the indirect effects of the genetic proxies for PCSK9 inhibitors on the outcomes mediated through Lp(a). We divided these estimates to the total effects of the genetic proxies of PCSK9 inhibitors on risk of CAD and IS and obtained the proportions of the effects mediated through lowering of Lp(a) levels. All data were analyzed using R statistical software version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

We detected 12 genetic variants in the vicinity of the *PCSK9* gene associated with lower LDL-C levels (Supplementary Table 1). Ten of these variants were also significantly ($<5x10^{-8}$) associated with lower plasma PCSK9 concentration (Supplementary Table 1), and thus fulfilled our criteria to be used as genetic proxies of PCSK9 inhibition. First, we explored the effects of genetically proxied PCSK9 inhibition on Lp(a) levels among 310,020 individuals from the UK Biobank. In the IVW MR analysis, we found 1-SD decrement in plasma PCSK9 concentration through variation in the *PCSK9* gene (corresponding to a 20.6 mg/dL [95%CI: −22.1 to −19.1] decrease in LDL-C levels) to be associated with a 3.8% decrease in log-Lp(a) levels (beta: −0.038, 95%CI: −0.053 to −0.023, $p = 2.0x10^{-6}$, Fig. 2), corresponding to a 0.582 mg/dL decrease in absolute Lp(a) levels (beta: 0.582, 95%CI: −0.928 to −0.236, $p = 0.001$, Supplementary Table 3). There was no evidence of heterogeneity in this analysis ($I^2 = 0\%$, $p = 0.93$) and the results were robust in sensitivity MR analyses with different underlying assumptions including the weighted median estimator, MR-Egger regression, and MR-PRESSSO (Fig. 2 and Supplementary Table 3). The intercept of MR-Egger regression was 0 ($p = 0.72$), thus implying no evidence of directional pleiotropy (Supplementary Table 3). MR-PRESSO detected no outliers. The reverse MR analysis showed no effect of genetically proxied Lp(a) levels on plasma PCSK9 concentration (beta per 1-SD increment in log-Lp(a) levels: 0.010, 95%CI: −0.008 to 0.028, $p = 0.287$), thus indicating a low risk of reverse causation. When weighing on LDL-C levels, the effects of genetically proxied PCSK9 inhibition on Lp(a) levels corresponded to a 6.6% reduction per 1-SD decrement in LDL-C levels (beta for log-Lp(a): −0.066, 95%CI: −0.092 to −0.040, $p = 3.9x10^{-6}$).

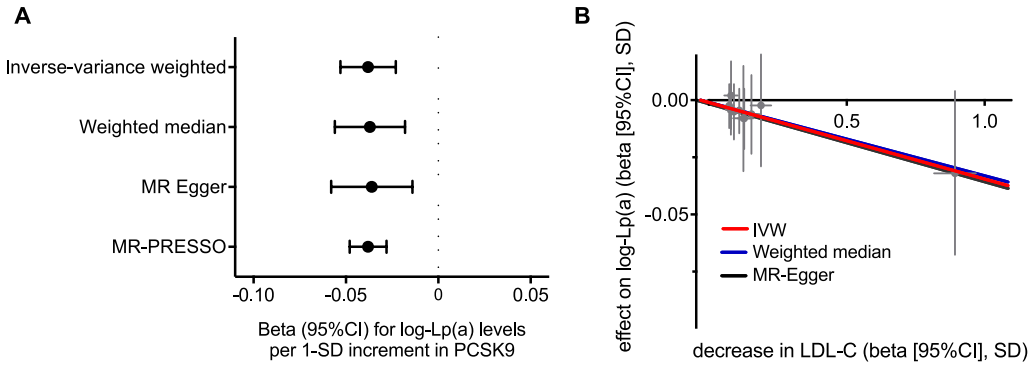

Fig. 2. Mendelian randomization associations between genetic proxies for PCSK9 inhibitors and log-transformed lipoprotein(a) (Lp(a)) levels.
(A) Associations between genetically proxied changes in PCSK9 concentration in plasma (1-SD decrement) and log-transformed Lp(a) levels, as derived from different Mendelian randomization methods. (B) Variant-specific effects on PCSK9 concentration in plasma and log-transformed Lp(a) levels and results from different Mendelian randomization methods.

Next, we explored the effects of the genetic proxies for PCSK9 inhibition on risk of CAD and IS in the CardioGramPLUSC4D and the MEGASTROKE Consortium, respectively. We found 1-SD decrement in plasma PCSK9 concentration to be also associated with lower risks for CAD (OR: 0.82, 95%CI: 0.76–0.88) and large artery atherosclerotic stroke (OR: 0.86, 95%CI: 0.75–0.98), but there were no significant associations with any IS (OR: 0.96, 95%CI: 0.91–1.02), cardioembolic stroke (OR: 1.00, 95%CI: 0.87–1.14), or small vessel stroke (OR: 1.06, 95%CI: 0.91–1.25, Supplementary Table 4). There was no evidence of heterogeneity in the associations of genetically proxied PCSK9 inhibition with CAD (I2 = 9.5%, $p = 0.36$) or large artery atherosclerotic stroke (I2 = 0%, $p = 0.87$) and MR-PRESSO detected no outlier single nucleotide polymorphisms. All sensitivity MR methods generated similar estimates and the Egger intercept was not significant, thus indicating no evidence of directional pleiotropy (Supplementary Table 4).

We then tested the associations between genetically proxied levels of Lp(a) with odds of cardiovascular outcomes in uni- and multivariable MR analyses. Genetically proxied Lp(a) levels through variation in the *LPA* gene were associated with a higher risk of CAD and large artery stroke when adjusting for their effects on plasma PCSK9 levels (Supplementary Table 5). Specifically, 1-log-increment in genetically proxied Lp(a) levels was associated with 22% higher odds for CAD (OR: 1.22, 95%CI: 1.17–1.27, $p = 1.7\text{x}10^{-21}$) and 14% higher odds for large artery

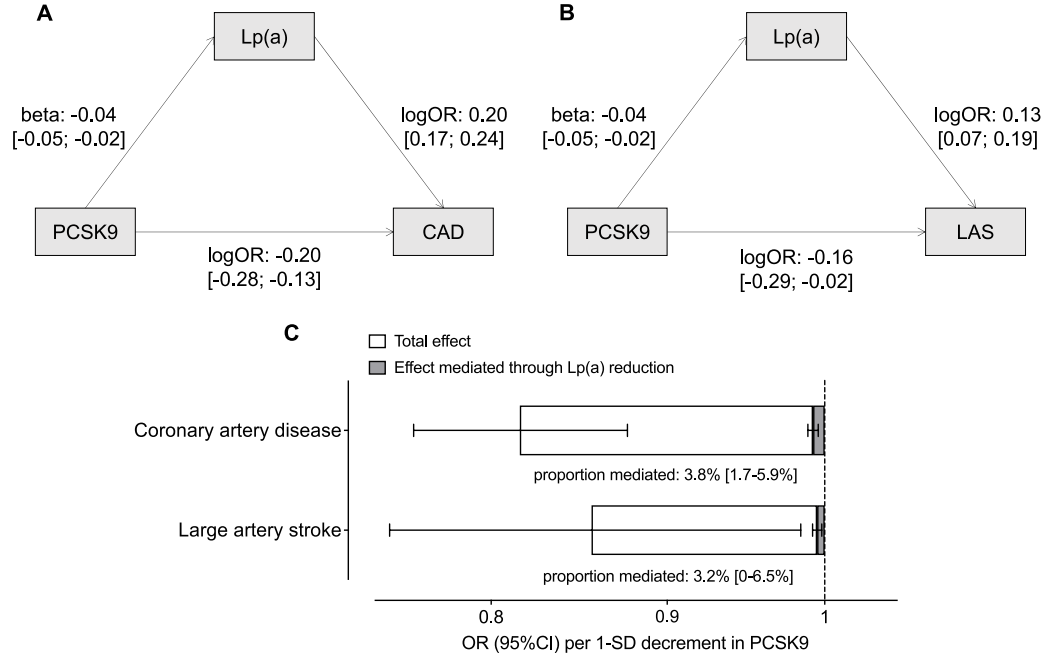

Fig. 3. Mediation Mendelian randomization analysis of the effects of genetic proxies for PCSK9 inhibitors on odds of coronary artery disease (CAD) and large artery stroke (LAS) mediated though Lp(a) reduction.
(A) Mendelian randomization analyses between genetically proxied PCSK9 concentration in plasma, log-transformed Lp(a) levels, and odds of CAD. The beta of the association between genetically proxied PCSK9 concentration in plasma and log-transformed Lp(a) levels, as well as the logOR of the associations between genetically proxied PCSK9 concentration in plasma and odds of CAD, is derived from inverse-variance weighted Mendelian randomization analysis. The logOR of the association between genetically proxied log-transformed Lp(a) levels and odds of CAD are derived from multivariable Mendelian randomization analyses adjusted for the effects of the variants affecting Lp(a) levels on PCSK9 concentration. (B) Similar analyses as in (A) for LAS. (C) Total Mendelian randomization effects of genetically proxied changes in PCSK9 concentration (1-SD decrement) on odds of CAD and LAS and indirect effects mediated through effects of genetically proxied PCSK9 concentration on log-transformed Lp(a) levels.

stroke (OR: 1.14, 95%CI: 1.08–1.21, $p = 4.3x10^{-4}$) when adjusting for the effects of the genetic proxies for Lp(a) on plasma PCSK9 (Supplementary Table 5). The results were stable across sensitivity MR analyses and similar results were obtained when using absolute and not log-transformed Lp(a) values (Supplementary Table 5).

Finally, we employed a mediation analysis framework to explore what proportion of the total effect of genetic proxies for PCSK9 inhibition on risk of CAD and large artery stroke could be mediated by the observed effects on Lp(a) levels (Fig. 3). We estimated that 1-SD decrement in plasma PCSK9 levels through variation in the genetic proxies for PCSK9 inhibition would lead to a 0.8% reduction in the odds for CAD (OR: 0.992, 95%CI: 0.989–0.995) and a 0.5% reduction in the odds for LAS (OR: 0.995, 95%CI: 0.992–0.998) due to reductions in Lp (a) levels. This corresponds to a proportion of 3.8% (95%CI: 1.7–5.9%) and 3.2% (95%CI: 0–6.5%) of the total effects of genetic proxies for PCSK9 inhibition on CAD and large artery stroke explained by Lp(a)-lowering, respectively (Fig. 3). All results were similar in sensitivity analyses using absolute Lp(a) values (mg/dL) instead of log-transformed Lp(a) values.

4. Discussion

In this MR study, we found that genetically proxied PCSK9 inhibition was associated with significantly lower Lp(a) levels (-4% in log-Lp(a) levels per 1-SD decrement in PCSK9 levels; corresponding to 21 mg/dl decrement in LDL-C levels). However, Lp(a) lowering contributed only modestly (3.8% and 3.2%) to the total effects of genetically proxied PCSK9 inhibitors on odds of CAD and large artery atherosclerotic stroke, respectively.

In the trials, the PCSK9 inhibitors alirocumab and evolocumab reduced Lp(a) levels by about 25% [27,28] or 14% per 1-SD decrement in LDL-C [27]. The Lp(a) level reduction associated with PCSK9 inhibition was more than twice as high in the PCSK9 trials than would be expected from the present MR study (14% *vs.* 7% per 1-SD decrement in LDL-C, respectively). This suggests that pharmacological PCSK9 inhibitors may lower Lp(a) levels through other off-target mechanisms not captured by genetic proxies for PCSK9 inhibitors. Indeed, alirocumab, rather than increasing Lp(a) clearance, was shown to blunt the secretion of Lp(a) from *in-vitro* human hepatocytes [29]. Lp(a) secretion is a complex, multi-step process increased by PCSK9 and attenuated by alirocumab [29]. Whether alirocumab inhibits Lp(a) secretion through mechanisms beyond mere PCSK9 inhibition is not known. Similarly to alirocumab, evolocumab monotherapy significantly decreased the synthesis of Lp(a) in 63 healthy men (-36%). When combined, *in-vivo*, with atorvastatin, evolocumab lowered Lp(a) by accelerating its catabolism (+59%) – likely to increased hepatic uptake – with no effect in Lp(a) synthesis [30]. The high proportion of patients on statins in the randomized trials on PCSK9 inhibitors – 89% in ODYSSEY OUTCOMES [31] and 99.7% in FOURIER [32] – may have amplified the Lp(a) lowering effects of alirocumab and evolocumab through increased hepatic Lp(a) uptake. In turn, this could explain the difference in Lp(a) lowering magnitude from our MR study, from which patients under lipid-lowering treatment at the time of Lp(a) measurement were excluded to avoid confounding in Lp(a) levels alterations due to pharmacological agents. In addition, ODYSSEY OUTCOMES and FOURIER focused on patients with established cardiovascular disease, who tended to have higher Lp (a) levels at baseline. For instance, in ODYSSEY OUTCOMES, median Lp (a) at baseline was 21.2 mg/dL[33], compared to 8.7 mg/dL in the general population [34]. In both trials, the absolute reduction in Lp(a) was greatest for individuals with higher baseline Lp(a) concentrations, and so was the risk reduction attributable to Lp(a) [8,9].

Concerning CAD, our results are consistent with a MR study that showed the effect of Lp(a) on the risk of CAD is log-linearly proportional to the absolute change in plasma Lp(a) levels [2]. Similarly to our study, the association between genetically proxied Lp(a) levels and CAD was independent of changes in LDL-C level owing to genetic variants that mimic the relationship of PCSK9 inhibitors with CAD risk. Large absolute reductions in Lp(a) by 100 mg/dL appeared to be required to produce clinically meaningful reductions in CAD risk [2]. Accordingly, in our study, the proportion of CAD risk reduction mediated through Lp(a) reduction was modest (3.6%), albeit statistically significant.

Concerning IS, an MR study from the Copenhagen General Population Study and Copenhagen City Heart Study showed an increased risk of IS with high levels of Lp(a). In our study, we assessed the IS subtypes and found a significant association only with large artery IS – not with cardioembolic and small vessel disease – supporting the atherogenic role of Lp(a) [35]. This is also in line with a recent large prospective study where elevated Lp(a) levels were associated with large artery atherosclerosis as stroke etiology [36].

Our study has strengths and limitations. Strengths included the large sample size of well characterized cohorts. Excluding patients on lipid-lowering therapies allowed to focus on the genetic proxies for PCSK9 inhibitors. Limitations are the limited comparability of our findings with FOURIER and ODYSSEY OUTCOMES, two secondary prevention trials that enrolled patients with established atherosclerosis. It is possible that Lp(a) lowering induced by PCSK9-inhibition reduces more recurrent than first-ever cardiovascular events. Second, we looked at CAD and IS separately, whereas in the trials the endpoint was composite, increasing statistical power. This may have emphasized cardiovascular protection associated with LDL-C reduction through genetically mediated PCSK9-inhibition. Last, it should be noted that our analyses were restricted to White British individuals, which may lead to limited generalizability.

In conclusion, genetic proxies for PCSK9 inhibitors are associated with lower Lp(a) levels. Yet, our data suggest that this effect could explain only a very small proportion of the overall association between genetic proxies for PCSK9 inhibitors and risk of CAD and IS. More targeted approaches designed to achieve large absolute reductions in Lp(a) among individuals with markedly elevated Lp(a) levels [7] might shed light on whether Lp(a) reduction could ultimately be an effective strategy for lowering vascular risk.

Financial support

This work was supported by the University Hospital Basel. The funder (University Hospital Basel) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. This study was performed and designed without the input or support of any pharmaceutical company or other commercial interest. MD is supported by the Deutsche Forschungsgemeinschaft (German Reuter Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology [EXC 2145 SyNergy – ID 390857198] and as part of CRC 1123 (B3) and DI 722/16-1. MKG was supported by a Walter-Benjamin fellowship from the German Reuter Foundation (Deutsche Forschungsgemeinschaft [DFG], GZ: GE 3461/1-1) and has received funding for this work from the FöFoLe program of LMU Munich (FöFoLe-Forschungsprojekt Reg.-Nr. 1120), the DFG Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198), and a Research Grant from the Fritz-Thyssen Foundation (Ref. 10.22.2.024MN).

CRediT authorship contribution statement

Gian Marco De Marchis: Conceptualization, Methodology, Supervision, Writing manuscript, Writing – review & editing. **Tolga D. Dittrich:** Writing manuscript, Writing – review & editing, Visualization. **Rainer Malik:** Writing manuscript, Writing – review & editing. **Annaelle V. Zietz:** Writing manuscript, Writing – review & editing. **Lilian F. Kriemler:** Writing manuscript, Writing – review & editing. **Brian A. Ference:** Writing manuscript, Writing – review & editing. **Martin Dichgans:** Writing manuscript, Writing – review & editing. **Marios K. Georgakis:** Conceptualization, Methodology, Data curation,

Formal analysis, Writing manuscript, Writing – review & editing.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: GMDM has been receiving support from the Swiss National Science Foundation (Nr 32003B_200573, Nr. PBBEP3_139388); Spezialprogramm Nachwuchsförderung Klinische Forschung, University of Basel; Science Funds (Wissenschaftspool) of the University Hospital Basel; Swiss Heart Foundation; ProPatient Foundation Basel; Bangerter-Rhyner-Stiftung; Swisslife Jubiläumsstiftung for Medical Research; Swiss Neurological Society; Fondazione Dr Ettore Balli; De Quervain research grant; Thermo Fisher GmbH; Novartis grant; travel honoraria by Bayer and BMS/Pfizer; speaker honoraria by Bayer and Medtronic; consultant honoraria by Bayer and Novartis. He is member of the Steering Committee of PACIFIC Stroke (NCT04304508). Industry payments are made to the research fund of the University Hospital Basel. TDD was or is supported by the Scientific Society Basel and the regulatory pool of the department of medicine of the University Hospital Basel. AVZ reports no competing interests. LFK reports no competing interests. BAF reports receiving research grants and consulting fees from Amgen, Sanofi, Regeneron, Novartis, and Ionis. MD reports no competing interests. MKG reports no competing interests.

Acknowledgments

We would like to thank the following consortia for providing data to the public: GLGC Consortium, CARDIoGRAMplusC4D Consortium, MEGASTROKE Consortium, and the deCODE genetic programs. This research was conducted with the help of UK Biobank Resource (UK Biobank application 2532, UK Biobank stroke study: developing a deep understanding of the determinants of stroke and its subtypes).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2022.09.007.

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