
There may be differences between this version and the published version. You are advised to consult the publisher’s version if you wish to cite from it.

http://eprints.gla.ac.uk/133541/

Deposited on: 2 October 2017
Genetic invalidation of Lp-PLA$_2$ as a therapeutic target:

large-scale study of five functional Lp-PLA$_2$ lowering alleles

John M. Gregson* PhD; Daniel F. Freitag* PhD; Praveen Surendran PhD; Nathan O. Stitziel MD PhD; Rajiv Chowdhury MD; Stephen Burgess PhD; Stephen Kaptoge PhD; Pei Gao PhD; James R. Staley MSc; Peter Willeit MD, PhD; Sune F. Nielsen PhD; Muriel Caslake PhD; Stella Trompet PhD; Linda M. Polfus PhD; Kari Kuulasmaa PhD; Jukka Kontto MSSc; Markus Perola MD, PhD; Stefan Blankenberg MD; Giovanni Veronesi PhD; Francesco Gianfagna MD, PhD; Satu Männistö; Akinori Kimura MD, PhD.; Honghuang Lin PhD; Dermot F. Reilly PhD; Mathias Gorski; Vladan Mijatovic on behalf of the CKDGen consortium; Patricia B. Munroe PhD; Georg B. Ehret MD on behalf of the International Consortium for Blood Pressure; Alex Thompson PhD; Maria Uria-Nickelsen PhD; Anders Malarstig PhD; Abbas Dehghan MD PhD on behalf of the CHARGE inflammation working group; Thomas F. Vogt PhD; Taishi Sasaoka MD, PhD; Fumihiko Takeuchi PhD; Norihiro Kato MD, DPhil; Yoshiji Yamada MD, PhD; Frank Kee MD; Martina Müller-Nurasyid PhD; Jean Ferrières MD; Dominique Arveiler MD; Philippe Amouyel MD; Veikko Salomaa MD, PhD; Eric Boerwinkle PhD; Simon G. Thompson FMedSci; Ian Ford PhD; J. Wouter Jukema MD; Naveed Sattar MD; Chris J. Packard PhD; Abdulla al Shafi Majumder MD; Dewan S Alam MD, PhD; Panos Deloukas PhD; Heribert Schunkert MD; Nilesh J. Samani FMedSci; Sekar Kathiresan MD on behalf of the MICAD Exome consortium; Børge G. Nordestgaard MD; Danish Saleheen* MD; Joanna M.M. Howson* PhD; Emanuele Di Angelantonio* MD; Adam S. Butterworth* PhD; John Danesh* FMedSci on behalf of the EPIC-CVD consortium and the CHD Exome* consortium

*denotes equal contribution; work was conducted at the University of Cambridge; Author’s affiliations are provided in the appendix

Correspondence: Dr Freitag or Professor Danesh

Department of Public Health and Primary Care
University of Cambridge
Strangeways Research Laboratory
Cambridge CB1 8RN
UK

john.danesh@phpc.cam.ac.uk
Tel: +44 1223 748 655
ABSTRACT (248 words)

Aims: Darapladib, a potent inhibitor of lipoprotein-associated phospholipase A\(_2\) (Lp-PLA\(_2\)), has not reduced risk of cardiovascular disease outcomes in recent randomized trials. We aimed to test whether Lp-PLA\(_2\) enzyme activity is causally relevant to coronary heart disease (CHD).

Methods: In 72,657 patients with CHD and 110,218 controls in 23 epidemiological studies, we genotyped five functional variants, four rare loss-of-function mutations (c.109+2T>C[rs142974898], Arg82His[rs144983904], Val279Phe[rs76863441], Gln287Ter[rs140020965]) and one common modest-impact variant (Val379Ala[rs1051931]) in PLA2G7, the gene encoding Lp-PLA\(_2\). We supplemented de-novo genotyping with information on a further 45,823 CHD patients and 88,680 controls in publicly available databases and other previous studies. We conducted a systematic review of randomized trials to compare effects of darapladib treatment on soluble Lp-PLA\(_2\) activity, conventional cardiovascular risk factors, and CHD risk with corresponding effects of Lp-PLA\(_2\)-lowering alleles.

Results: Lp-PLA\(_2\) activity was decreased by 64% (p=2.4×10\(^{-25}\)) with carriage of any of the four loss-of-function variants, by 45% (p<10\(^{-300}\)) for every allele inherited at Val279Phe, and by 2.7% (p=1.9×10\(^{-12}\)) for every allele inherited at Val379Ala. Darapladib 160mg once-daily reduced Lp-PLA\(_2\) activity by 65% (p<10\(^{-300}\)). Causal risk ratios for CHD per 65% lower Lp-PLA\(_2\) activity were: 0.95 (0.88-1.03) with Val279Phe; 0.92 (0.74-1.16) with carriage of any loss-of-function variant; 1.01 (0.68-1.51) with Val379Ala; and 0.95 (0.89-1.02) with darapladib treatment.

Conclusions: None of a series of Lp-PLA\(_2\)–lowering alleles was related to CHD risk, suggesting that Lp-PLA\(_2\) is not a valid therapeutic target.

KEY WORDS: Human genetics, target validation, coronary heart disease, Lipoprotein-associated phospholipase A\(_2\), darapladib
ABBREVIATIONS

CHD = Coronary Heart Disease
CI = Confidence Interval
HDL = High-density lipoprotein
LDL = Low-density lipoprotein
Lp-PLA₂ = Lipoprotein-associated phospholipase A₂
MI = Myocardial infarction
SD = Standard deviation

Word count: 3003 (excluding references and abstract (248 words))

3 main figures, 2 tables, 1 Appendix
Supplement (comprising a supplementary note and 6 tables, 4 figures)
INTRODUCTION

Lipoprotein-associated phospholipase A2 (Lp-PLA2), an enzyme expressed by inflammatory cells in atherosclerotic plaques, is carried in the circulation bound predominantly to low-density-lipoprotein (LDL)\(^1\). Lp-PLA2 (also called platelet-activating factor acetyl hydrolase) hydrolyzes oxidized phospholipids to yield pro-inflammatory products implicated in endothelial dysfunction, plaque inflammation, and formation of necrotic core in plaque\(^1\). Observational\(^2\) and experimental studies in humans and animals have suggested that Lp-PLA2 could be a valid therapeutic target, postulating this enzyme to link oxidative modification of LDL and development of inflammatory responses to arterial intima\(^1\). Previous studies have investigated genetic variants altering Lp-PLA2 function in relation to coronary heart disease (CHD) risk\(^3, 4\). However, these studies have generally yielded inconclusive, or conflicting results\(^3, 4\), perhaps due to limited statistical power and to limited knowledge about variants altering Lp-PLA2 function (i.e., previous studies have been able to consider only one loss-of-function variant in \(PLA2G7\), the gene encoding Lp-PLA2).

However, two phase 3 randomized trials of darapladib, a potent inhibitor of Lp-PLA2 activity, have not shown reductions in cardiovascular risk\(^5, 6\). These results could, at least in part, have been due to features of the trials. One of the phase 3 trials was restricted to patients recently hospitalized with acute coronary syndromes\(^5\), yet many cardiovascular events occurring early after acute coronary syndromes may relate to thrombotic mechanisms and not be modifiable through Lp-PLA2 inhibition. Trials used statins as background therapy, so any Lp-PLA2 inhibition achieved with statins could have reduced any incremental benefits of darapladib. Trials could not assess the effects of prolonged Lp-PLA2 inhibition because they recorded only about 3-4 years of median follow-up\(^5, 6\).

An alternative explanation is that darapladib did not reduce cardiovascular risk because Lp-PLA2 is not a causal risk factor in cardiovascular disease. We tested this possibility by investigating natural loss of Lp-PLA2 activity. Studies of Lp-PLA2–lowering alleles should complement randomized trials of darapladib because genotypes are fixed at conception, avoiding potential distorting effects of pre-existing disease and medication usage. Furthermore, Lp-PLA2–lowering alleles should produce lifelong, rather than shorter-term, Lp-PLA2 inhibition.

In over 260,000 participants of European, South Asian, or East Asian ancestries, we studied five functional variants in \(PLA2G7\). We compared effects of Lp-PLA2–lowering alleles on soluble Lp-PLA2 activity, conventional cardiovascular risk factors, and CHD risk with corresponding effects of darapladib, using results from randomized trials.
METHODS

Study design

Figure 1 summarises the study approach. Table 1 provides definitions and sources of data used. First, we identified four loss-of-function mutations and one missense variant in PLA2G7 suggested by previous experimental and bioinformatics studies, thereby developing an allelic series for Lp-PLA2 activity. Second, we assessed associations of these variants — both singly and in combination — with soluble Lp-PLA2 activity, conventional cardiovascular risk factors, and CHD risk in people of European, South Asian, or East Asian continental ancestries. Third, we compared associations of Lp-PLA2–lowering alleles with the aforementioned traits and CHD risk with the effects of darapladib treatment through a systematic review of randomized trials.

Genetic variants

We defined loss-of-function variants as non-synonymous variants with in vitro or in vivo evidence demonstrating complete lack of Lp-PLA2 activity or sequence changes expected to abolish Lp-PLA2 function (e.g., nonsense variants or mutations in essential splice sites). We selected variants through a systematic search for loss-of-function variants using the UniProt database7, the Exome Aggregation Consortium database (Cambridge, MA, USA; URL: http://exac.broadinstitute.org; [accessed November 2014])8, studies of site-directed mutagenesis9–11 and results from targeted gene sequencing12. Among the full set of variants identified (eTable 1), we selected the following variants that could be detected in the 1000 Genomes13 or the Exome sequencing14 projects (and, hence, potentially studied at the population level): the splice site mutation 109+2T>C (rs142974898); two non-synonymous variants — Arg82His (rs144983904) and Val279Phe (rs76863441); and the nonsense variant Gln287Ter (rs140020965). These loss-of-function variants are rare in European and South Asian ancestry populations, whereas carriage of 279Phe is common in East Asian ancestry populations and abolition of Lp-PLA2 activity is well documented15. Additionally, we studied Val379Ala (rs1051931), a functional variant common in European ancestry populations, which lowers Lp-PLA2 activity only modestly9,16, in contrast with the substantial Lp-PLA2–lowering achieved by the loss-of-function variants described above.

Samples and data for genetic studies

We aimed to maximise study power and comprehensiveness by using the following complementary approaches to generate new data on, as well as to collate systematically existing relevant information about, the PLA2G7
variants mentioned above: (1) we conducted de-novo genotyping for 72,657 CHD patients and 110,218 controls (the majority of whom also had information available on some cardiovascular risk factors); (2) we accessed non-overlapping summary-level data from the only known global genetics consortium of CHD\textsuperscript{17}, yielding information on a further 35,735 CHD patients and 73,481 controls; (3) we conducted a systematic review (supplemented by provision of tabular data from each study investigator) of published East Asian CHD studies of Val279Phe because these studies were not represented in the global CHD consortium, yielding information on a further 10,088 CHD cases and 15,199 controls; (4) we accessed summary-level data from the largest available global genetics consortium on each of several relevant cardiovascular risk factors (eg, Lp-PLA\textsubscript{2} activity, conventional lipids, blood pressure), yielding information on 489,045 participants. Each of these sources of information is summarised below and in Table 1.

Coronary heart disease outcomes For CHD outcomes, we had access to data for a total of 92,995 patients and 162,228 controls. For 182,875 of these participants (72,657 CHD patients, 110,218 controls), we did de-novo genotyping of the four loss-of-function variants (c.109+2T>C, Arg82His, Val279Phe, Gln287Ter) and Val379Ala using customized Exome arrays (Illumina, California, USA) by technicians masked to the phenotypic status of the participants’ samples. For 35,829 CHD cases, 44,948 controls in eight studies, we had access to individual-participant data. The eight studies were: the Bangladesh Risk of Acute Vascular Events Study (BRAVE)\textsuperscript{18}, Copenhagen City Heart Study (CCHS)\textsuperscript{19}, Copenhagen Ischemic Heart Disease/Copenhagen General Population Study (CIHDS/CGPS)\textsuperscript{19}, European Prospective Investigation into Cancer and Nutrition-Cardiovascular Disease Study (EPIC-CVD)\textsuperscript{20}, MONICA Risk, Genetics, Archiving, and Monograph (MORGAM) study\textsuperscript{21, 22}, Pakistan Risk of Myocardial Infarction Study (PROMIS)\textsuperscript{23}, Pravastatin in elderly individuals at risk of vascular disease (PROSPER) trial\textsuperscript{24} and the West of Scotland Coronary Prevention Study (WOSCOPS)\textsuperscript{25} (these eight studies are collectively called the “CHD Exome+ consortium”). For 15 additional studies (collectively called the “MICAD consortium”), we used similar genotyping methods to those described above but did not genotype c.109+2T>C and had access only to study-level data. We supplemented de-novo data on Val379Ala with non-overlapping consortium-level results from a further 35,735 CHD patients and 73,481 controls in the transatlantic Coronary Artery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM)\textsuperscript{26} and Coronary Artery Disease Genetics (C4D)\textsuperscript{27} consortia (Table 1). We obtained tabular data on Val279Phe from seven East Asian studies involving a total of 10,088 CHD cases and 15,199 controls, identified through systematic review (eTable 5 and Supplement). About 90% of CHD patients in our genetic
analysis had myocardial infarction or other major acute coronary events; the remainder had angiographic
evidence alone (eg, >50% coronary stenosis, eTables 2 & 5).

Lp-PLA₂ activity For 13,835 participants, we had information on functional variants in PLA2G7 and Lp-PLA₂
activity, using data from de-novo genotyping in MORGAM²¹,²² and PROSPER²⁴, supplemented by published
data from the CHARGE Consortium (ie, from the Atherosclerosis Risk in Communities [ARIC] Study²⁸,
Cardiovascular Health Study¹⁶, Framingham Heart Study¹⁶, and Rotterdam study¹⁶), and from 12 East Asian
studies identified through the systematic review described above (Table 1, Supplement & eFigure1, eTables
2-3).

Conventional cardiovascular risk factors For 177,343 participants, we had information on functional variants in
PLA2G7 and conventional cardiovascular risk factors and several other traits, including circulating
concentrations of LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, insulin, and C-reactive protein, and
values of systolic and diastolic blood pressure, body-mass index, and estimated glomerular filtration rate. Again,
we supplemented data from our de-novo genotyping, with information from existing global genetics consortia
(Table 1, eTables 2-4).

Randomized trials of darapladib

To compare genetic associations with effects of pharmacological Lp-PLA₂ inhibition, we conducted a
systematic review to identify randomized placebo-controlled trials of darapladib that had reported on Lp-PLA₂
activity, conventional risk factors, and/or CHD events (Supplement). CHD events in the trials were defined as
fatal CHD, MI or urgent revascularisation, as recorded in STABILITY (Stabilization of Atherosclerotic Plaque
by Initiation of Darapladib Therapy) and in SOLID-TIMI 52 (Stabilization of Plaque Using Darapladib-
Thrombolysis in Myocardial Infarction 52)⁵,⁶. We pooled results across trials by fixed-effect inverse-variance
weighted meta-analysis (eFigures 2&3; see Supplement for details of the methods used).

Statistical methods

We defined effect alleles as those associated with lower Lp-PLA₂ activity and assumed an additive model. For
participant-level data, we assessed associations of Lp-PLA₂-lowering alleles with CHD using the genome-wide
efficient mixed model analysis, an approach that models each genetic variant as a fixed-effect, but includes both
fixed-effect and random-effects of genetic inheritance²⁹ to account for population stratification and relatedness
among participants (Supplement). The four rare loss-of-function variants were tested jointly within each study
by counting the number of loss-of-function alleles carried by each participant. Log odds ratios and standard
ersors were meta-analysed across studies using fixed-effect meta-analysis. For studies contributing only study-
level data, we performed a similar test by conducting a combined burden test across studies using the R package
seqMeta v1.2 (http://cran.r-project.org/web/packages/seqMeta/).

We calculated associations of Lp-PLA₂-lowering alleles with soluble Lp-PLA₂ activity and conventional risk
factors using linear regression within each study, and then combined the regression coefficients using fixed-
effect meta-analysis. When data were missing, we used information on rs1805018 as a proxy for Val279Phe and
information on rs7756935 or rs3799277 as proxies for Val379Ala (Supplement). To account for population
stratification, we adjusted for the first principal component of ancestry (Supplement). We calculated risk ratios
for CHD with decrements in Lp-PLA₂ activity, dividing the log transformed risk ratio and confidence interval
(CI) by the effect on Lp-PLA₂ activity of the instrument (ie, the genetic variant)³⁰. We investigated
heterogeneity using the $I^2$ statistic. We used Stata 13.1.
RESULTS

Of the 261,950 total participants in this analysis, we studied 195,715 individuals of European ancestry, 34,221 individuals of South Asian ancestry, and 32,014 individuals of East Asian ancestry. In people of European or South Asian ancestry without CHD, the frequency of alleles in PL2G7 that lower Lp-PLA₂ activity was 0.005% at c.109+2T>C, 0.04% at Arg82His, 0.04% at Val279Phe, and 0.025% at Gln287Ter (i.e., in aggregate, 0.2% of the European or South Asian participants in the current study carried one of these loss-of-function alleles, although no one carried more than one of these variants), and about 80% at Val379Ala. In people of East Asian ancestry without CHD, the frequency of Val279Phe was about 15% and about 2% of the individuals were homozygous carriers of the 279Phe allele.

Soluble Lp-PLA₂ activity

Compared with non-carriers, homozygote carriers of the 279Phe allele had 94% lower Lp-PLA₂ activity (p<10⁻³⁰⁰). For each 279Phe allele inherited, Lp-PLA₂ activity decreased by 45% (1.59 SD, 95% CI: 1.61-1.57; p<10⁻³⁰⁰). In Europeans who inherited any one of the four rare Lp-PLA₂ loss-of-function alleles, Lp-PLA₂ activity decreased by 64% (2.25 SD, 2.68-1.83; p=1.6×10⁻²⁵). For each 379Ala allele inherited, Lp-PLA₂ activity decreased by 2.7% (0.096 SD, 0.122-0.069; p=1.9×10⁻¹²). By comparison, 160mg once-daily darapladib reduced Lp-PLA₂ activity by 65% (2.26 SD, 2.31-2.21; p<10⁻³⁰⁰). Study-level estimates are provided in eFigure 2.

Cardiovascular risk factors

None of the Lp-PLA₂–related variants we studied was significantly associated with values of LDL-cholesterol, HDL-cholesterol, triglycerides, systolic or diastolic blood pressure, body-mass index, estimated glomerular filtration rate, glucose, insulin, and C-reactive protein (Figure 2). By comparison, in previous randomized placebo-controlled trials, darapladib did not significantly affect concentrations of LDL-cholesterol or log triglycerides, but could have slightly increased systolic blood pressure and HDL-cholesterol values and slightly decreased C-reactive protein concentration (Figure 2).
Clinical CHD outcomes

Compared to non-carriers, the odds ratio for CHD was 0.99 (0.95-1.03) in 279Phe heterozygotes, and 0.93 (0.82–1.05) in 279Phe homozygotes (i.e. nearly complete loss of Lp-PLA2 function: Figure 3). For each loss-of-function (279Phe) allele inherited, the odds ratio for CHD was 0.97 (0.91-1.02; $I^2=30\%$; $P_{\text{Heterogeneity}}=0.2$). In Europeans and South Asians who inherited one of the four rare Lp-PLA2-loss-of-function alleles, the odds ratio for CHD was 0.92 (0.74-1.16; $I^2=0\%$; $P_{\text{Heterogeneity}}=0.8$; Figure 3). For each 379Ala allele inherited, the odds ratio for CHD was 1.00 (0.98-1.02; $I^2=0.0\%$; $P_{\text{Heterogeneity}}=0.5$; Figure 3). Study-level results are provided in eFigure 3. In sensitivity analyses, odds ratios with each loss-of-function variant were similar to the odds ratio that combined information across the four loss-of-function variants we studied. There was no evidence of heterogeneity in odds ratios between European and South Asian ancestry populations (eFigure 4).

Genetic risk ratios for CHD per 65% lower Lp-PLA2 activity (i.e. the reduction achievable with darapladib treatment) were: 0.95 (0.88-1.03) with Val279Phe in East Asians; and 0.92 (0.74-1.16) with carriage of any one of the four rare variants studied in Europeans and South Asians; and 1.01 (0.68-1.51) with Val379Ala (Table 2).

By comparison, the risk ratio for CHD with darapladib treatment (i.e. also per 65% lower Lp-PLA2 activity) was 0.95 (0.89-1.02; Table 2).
DISCUSSION

In 2008, GlaxoSmithKline launched a ~$1 billion program of phase 3 trials of darapladib, a compound which did not reduce cardiovascular event rates in two secondary prevention trials. We tested whether Lp-PLA2 enzyme activity is causally relevant to CHD by studying five functional alleles that produce widely differing degrees of reduction in Lp-PLA2 activity. We found that none was related to CHD risk. Hence, our large-scale human genetic data, which are concordant with results from phase 3 trials, suggest that Lp-PLA2 enzyme activity is not causally relevant to CHD. The implication is that darapladib did not reduce CHD risk in recent trials principally because Lp-PLA2 is not a valid therapeutic target.

Three features of our study merit comment. First, we studied almost 20 times more CHD patients than the previous largest study of loss-of-function PLA2G7 alleles, thereby providing the first robust genetic evaluation of effect sizes of Lp-PLA2 inhibition relevant to phase 3 trials such as relative risk reductions for CHD of 20%. For example, for the Val279Phe variant we had >99% power to detect a 20% risk reduction in CHD for a 65% genetic reduction in Lp-PLA2 activity (ie, an effect on Lp-PLA2 activity similar to that achieved by darapladib).

Second, our study has provided the first investigation in CHD of a series of functional alleles that each reduce Lp-PLA2 function via different molecular mechanisms. Specifically, we studied five different Lp-PLA2-lowering alleles: three of the alleles were coding variants that produced different amino acid substitutions; two of the alleles produced protein truncations (one due to a nonsense mutation; the other due to a splice-site mutation). Because we observed null and broadly concordant findings for CHD risk across these alleles that each changed the enzyme in a different way, we can more confidently conclude there is no material cause-and-effect relationship. By contrast, when the initial phase 3 trial of darapladib was launched in 2008, only two of the five alleles we studied had yet been identified: data on Val379Ala, a weak effect missense variant, were inconclusive because CHD studies were under-powered; data on Val279Phe, a loss-of-function variant, and CHD risk were sparse and restricted to East Asian populations.

A third feature was our study’s analysis of substantial data from three different major ethnic groups: Europeans, South Asians, and East Asians. This ethnic diversity enhanced the generalisability of our results.

Our study had potential limitations. We used the “major coronary events” endpoint from phase 3 darapladib trials for comparison of pharmacological inhibition of Lp-PLA2 with genetic inhibition, whereas definitions of CHD used in human genetic studies were not strictly uniform (eg, some studies included patients with only...
angiographic evidence of CHD). However, about 90% of CHD cases included in our genetic analyses had myocardial infarction or other major acute coronary events.

It could be that cardioprotective benefits of Lp-PLA₂ inhibition were obscured by pleiotropic effects of PLA2G7 variants; for example, 279Phe is known to produce a misfolded version of Lp-PLA₂ not secreted by cells, prompting suggestions that its carriage could produce “off-target” effects such as increased cell death\cite{32, 33}. However, because we found null associations between four other functional alleles in PLA2G7 and CHD, each of which operates via a different molecular mechanism, it argues against this explanation.

Lifelong genetic reductions in Lp-PLA₂ could result in compensatory responses that increase CHD risk. However, this explanation seems unlikely because it would require any such compensation to apply similarly across alleles that produce widely differing degrees of reduction in Lp-PLA₂ activity. Furthermore, any such compensation could not operate through known cardiovascular mechanisms because we observed no associations between Lp-PLA₂-lowering alleles and several established and emerging cardiovascular risk factors.

Soluble enzyme activity could be an imperfect indicator of the relevance of Lp-PLA₂ to atherosclerotic plaques. However, for homozygote carriers of 279Phe, Lp-PLA₂ activity should be almost abolished across all tissues. Finally, we studied life-long genetic reductions in Lp-PLA₂ activity in relation to first-onset CHD outcomes rather than recurrent CHD, whereas darapladib trials studied recurrent coronary events in patients with stable or acute coronary disease.

Our findings suggest that, in retrospect, the lack of efficacy of darapladib in phase 3 trials could, in principle, have been anticipated. However, it is important to acknowledge that the methods and data used in the current analysis were not available at the time the darapladib program was launched. Nevertheless, the current data underscore the growing importance of human genetic approaches to enhance the efficiency of development of medicines by validating (or invalidating) novel drug targets\cite{34-37}. Our results also illustrate how human genetic evidence can assist interpretation of observational epidemiological data. We found that functional alleles in PLA2G7 do not alter levels of pro-atherogenic lipids (eg, LDL-C). This result suggests that such pro-atherogenic lipids do not mediate associations between Lp-PLA₂ activity and CHD, supporting the need to adjust epidemiological associations of Lp-PLA₂ activity with CHD risk for pro-atherogenic lipids (an approach which yields results consistent with non-causality)\cite{2}. 

12
In summary, none of a series of Lp-PLA$_2$–lowering alleles was related to CHD risk, suggesting that Lp-PLA$_2$ is not a valid therapeutic target.
ACKNOWLEDGEMENTS

The work of the coordinating center was funded by the UK Medical Research Council (G0800270), British Heart Foundation (SP/09/002), British Heart Foundation Cambridge Cardiovascular Centre of Excellence, UK National Institute for Health Research Cambridge Biomedical Research Centre, European Research Council (268834), European Commission Framework Programme 7 (HEALTH-F2-2012-279233). The Supplement includes a list provided by investigators of some of the funders of the component studies in this analysis.

DISCLOSURES

Anders Malarstig and Maria Uria-Nickelsen are full time employees of Pfizer. Since October 2015, Daniel Freitag has been a full time employee of Bayer. The funders had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, and in the preparation, review, or approval of the manuscript.


Europeans reveals several rare loss-of-function mutations. *Pharmacogenomics J*


2012;491(7422):56-65.


2012;337(6090):64-69.


17. Kulathinal S, Karvanen J, Saarela O, Kuulasmaa K. Case-cohort design in practice - experiences from the MORGAM Project. *Epidemiol Perspect Innov*

2007;4:15.


2010;210(1):28-34.


2015.


38. Daida H, Iwase T, Yagi S, Ando H, Nakajima H. Effect of darapladib on plasma lipoprotein-
associated phospholipase A2 activity in Japanese dyslipidemic patients, with exploratory

39. Randall JC, Winkler TW, Katalik Z, Berndt SI, Jackson AU, Monda KL, Kilpelainen TO, Esko T,
Magi R, Li S, Workalemahu T, Feitosa MF, Croteau-Chonka DC, Day FR, Fall T, Ferreira T,
Gustafsson S, Locke AE, Mathieon I, Scherag A, Vedantam S, Wood AR, Liang L,
Steinhorsdottir V, Thorleifsson G, Dermitzakis ET, Dimas AS, Karpe F, Min JL, Nicholson G,
JJ, Prokopenko I, Waite LL, Harris TB, Smith AV, Shuldiner AR, McArdle WL, Caulfield MJ,
Munroe PB, Gronberg H, Chen YD, Li G, Beckmann JS, Johnson T, Thorsteinsdottir U, Teder-
Laving M, Khaw KT, Wareham NJ, Zhao JH, Amin N, Oostra BA, Kraja AT, Province MA, Cupples
LA, Heard-Costa NL, Kaprio J, Ripatti S, Surakka I, Collins FS, Saramies J, Tuomilehto J, Jula A,
Salomaa V, Erdmann J, Hengstenberg C, Loley C, Schunkert H, Lamina C, Wichmann HE,
Albrecht E, Gieger C, Hicks AA, Johansson A, Pramstaller PP, Kathiresan S, Speliotes EK, Penninx
B, Hartikainen AL, Jarvelin MR, Gyllensten U, Boomsma DI, Campbell H, Wilson JF, Chanock SJ,
Farrall M, Goel A, Medina-Gomez C, Rivadeneire F, Estrada K, Uitterlinden AG, Hofman A,
Ohlsson C, Eklund N, Eriksson JG, Barlassina C, Rivolta C, Nolte IM, Snieder H, Van der Klauw
MM, Van Vliet-Oostapchouk JV, Gejman PV, Shi J, Jacobs KB, Wang Z, Bakker SJ, Mateo L, I,
Navis G, van der Harst P, Martin NG, Medland SE, Montgomery GW, Yang J, Chasman DI,
Ridker PM, Rose LM, Lehtimaki T, Raitakari O, Absher D, Iribarren C, Basart H, Hovingh KG,
Hypponen E, Power C, Anderson D, Beilby JP, Hui J, Jolley J, Sager H, Bornstein SR, Schwarz PE,
Bolton JL, Fowkes G, Fraser RM, Price JF, Fischer K, Krijuta KK, Metspalu A, Mihailov E,
Langenberg C, Luan J, Ong KK, Chines PS, Rivadeneire F, Estrada K, Uitterlinden AG, Hofman A,
Ohlsson C, Eklund N, Eriksson JG, Barlassina C, Rivolta C, Nolte IM, Snieder H, Van der Klauw
MM, Van Vliet-Oostapchouk JV, Gejman PV, Shi J, Jacobs KB, Wang Z, Bakker SJ, Mateo L, I,
Navis G, van der Harst P, Martin NG, Medland SE, Montgomery GW, Yang J, Chasman DI,
Ridker PM, Rose LM, Lehtimaki T, Raitakari O, Absher D, Iribarren C, Basart H, Hovingh KG,
Hypponen E, Power C, Anderson D, Beilby JP, Hui J, Jolley J, Sager H, Bornstein SR, Schwarz PE,
Bolton JL, Fowkes G, Fraser RM, Price JF, Fischer K, Krijuta KK, Metspalu A, Mihailov E,
Langenberg C, Luan J, Ong KK, Chines PS, Rivadeneire F, Estrada K, Uitterlinden AG, Hofman A,
Ohlsson C, Eklund N, Eriksson JG, Barlassina C, Rivolta C, Nolte IM, Snieder H, Van der Klauw
MM, Van Vliet-Oostapchouk JV, Gejman PV, Shi J, Jacobs KB, Wang Z, Bakker SJ, Mateo L, I,
Navis G, van der Harst P, Martin NG, Medland SE, Montgomery GW, Yang J, Chasman DI,
Ridker PM, Rose LM, Lehtimaki T, Raitakari O, Absher D, Iribarren C, Basart H, Hovingh KG,
Hypponen E, Power C, Anderson D, Beilby JP, Hui J, Jolley J, Sager H, Bornstein SR, Schwarz PE,
Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YP, O’Connell JR, Steinle
Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Bellby JP, Lawrence RW, Clarke R,
Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kahonen M, Viikari J, Adair LS, Lee
NR, Chen MH, Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Mooser V, Chaturvedi N,
Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grassler J, Groop L,
I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ,
Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR,
Soler AM, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL,
Wiggins KL, Doumataye A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V,
Langefeld CD, Rosangren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie
CA, Salako T, Iwai N, Kita Y, Ohigara T, Ohkubo T, Okamura T, Ueshima H, Umemura S,
O’Donnell CJ, Schwartz JM, Ikram MA, Longstreth WT, Jr., Mosley TH, Seshadri S, Shrine NR,
Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE,
KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isacca A, Rivadeneira F,
T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P, Hercberg S, Lathrop M,
Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G. Genetic variants in novel pathways

41. Willer CJ, Schmidt MD, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J,
Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog
RM, Freiatt DF, Gurudasani D, Heikkila K, Hyyponen E, Isaacs A, Jackson AU, Johansson A,
Johnson T, Kaakin M, Kettunen J, Kliber ME, Li X, Luan J, Lyytikainen LP, Magnusson PK,
Mangino M, Mihailov E, Montasser ME, Muller-Nurasyid M, Nolte IM, O’Connell JR, Palmer CD,
Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C,
Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF,
Bonnycastle LL, Brambilla P, Burnett MS, Cesa R, Dimitriou M, Doney A, Elliott P, Epstein SE,
Eyljofsson GI, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans
G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Iliig T, Jones MR,
Lehtimaki T, Lin SY, Lindstrom J, Loos RJ, Mach F, Mc Ardle WL, Meisenger C, Mitchell BD,
Muller G, Nagaraja R, Narisu N, Nieminen TV, Nsbuga RN, Olafsson I, Ong K, Palotie A,
Pamamarkou T, Pomilla C, Poula A, Rader DJ, Reilly PM, Ridker PM, Rivadeneira F, Rudan I,
Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild Sh,
Willemse G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL,
Bandingelli S, Bennett F, Bochud M, Boomsma DJ, Borecki IB, Bornstein SR, Bovet P,
Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YD, Collins FS, Cooper RS, Danesh J,
Dedoussis G, de FU, Fernanil AB, Ferriere J, Ferrucci L, Freimer N, Gieger C, Groop LC,
Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A,
Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Jarvelin MR, Jula A,
Kahonen M, Kaprio J, Kesaniemi A, Kivimaki M, Kooneer JS, Koudstaal PJ, Krauss RM, Kuhl D,
Kuusisto J, Kyyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, Marz W, McCarthy
MI, McKenzie CA, Meneton P, Metspalu A, Molianen L, Morris AD, Munroe PB, Njolstad I,
Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quenrmeous T, Rauramaa R,
42. Dehghan A, Dupuis J, Barbatic M, Bis JC, Eiriksdottir G, Lu C, Pellikka N, Wallaschofski H,
Kettunen J, Henneman P, Baumert J, Strachan DP, Fuchsberger C, Vitart V, Wilson JF, Pare G,
Ry, Schnabel RB, Nambi V, Kavousi M, Ripatti S, Nauck M, Smith NL, Smith AV, Sundvall J,
Tracy RP, Launer LJ, Buring JE, Yamamoto JF, Folsom AR, Sijbrands EJ, Pankow J, Elliott P,
Keaney JF, Sun W, Sarin AP, Fontes JD, Badola S, Astor BC, Hofman A, Pouta A, Werdan K,
Greiser KH, Kuss O, Meyer zu Schwabedissen HE, Thiery J, Jamshidi Y, Nolte IM, Soranzo N,
Spector TD, Volzke H, Parker AN, Aspelund T, Bates D, Young L, Tsui K, Sicoviski DS, Guo X,
Rotter JI, Uda M, Schlessinger D, Rudan I, Hicks AA, Penninx BW, Thorand B, Gieger C, Coresh J,
Willemsen G, Harris TB, Uitterlinden AG, Jarvelin MR, Rice K, Radke D, Salomaa V, Willems van
DK, Boerwinkle E, Vasan RS, Ferrucci L, Gibson QD, Bandinelli S, Snieder H, Boomsma DI, Xiao
X, Campbell H, Hayward C, Pramstaller PP, van Duijn CM, Peltonen L, Psaty BM, Gudnason V,
Ridker PM, Homuth G, Koenig W, Ballantyne CM, Witteman JC, Benjamin EJ, Perola M,
Chasman DI. Meta-analysis of genome-wide association studies in >80 000 subjects identifies

AUTHOR CONTRIBUTIONS:
FIGURE LEGENDS

Figure 1: Summary of study design

Figure 2: Mean per allele differences in Lp-PLA₂ activity and cardiovascular risk factor levels by Lp-PLA₂ lowering alleles or with darapladib 160mg daily

Figure 3: Association of Lp-PLA₂ lowering alleles with Lp-PLA₂ activity and CHD risk
APPENDIX

List of authors and affiliations

John M. Gregson* PhD, Daniel F. Freitag* PhD, Praveen Surendran PhD, Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; Nathan O. Stitziel MD PhD, Departments of Medicine and Genetics, Washington University School of Medicine, St Louis, Missouri 63110, USA; Rajiv Chowdhury MD, Stephen Burgess PhD, Stephen Kaptoge PhD, Pei Gao PhD, James R. Staley MSc, Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; Peter Willeit MD, PhD, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK and Department of Neurology, Innsbruck Medical University, Innsbruck, Austria; Sune F. Nielsen PhD, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark; Muriel Caslake PhD, University of Glasgow, Glasgow, UK; Stella Trompet PhD, Leiden University Medical Center, Leiden, Netherlands; Linda M. Polfus PhD, University of Texas Health Science Center Houston, TX, USA; Kari Kuulasmaa PhD, Jukka Kontto MSSc, THL-National Institute for Health and Welfare, Helsinki, Finland; Markus Perola MD, PhD, Institute of Molecular Medicine FIMM, University of Helsinki and Department of Health, National Institute for Health and Welfare, Helsinki, Finland; Stefan Blankenberg MD, Department of General and Interventional Cardiology, University Heart Center Hamburg, Germany and University Medical Center Hamburg Eppendorf, Hamburg, Germany; Giovanni Veronesi PhD, EPIMED Research Center, Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy; Francesco Gianfagna MD, PhD, EPIMED Research Center, Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy and Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy; Satu Männistö, THL-National Institute for Health and Welfare, Helsinki, Finland; Akinori Kimura MD, PhD, Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University (TMDU), Tokyo, Japan; Honghuang Lin PhD, Section of Computational Biomedicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA and The NHLBI's Framingham Heart Study, Framingham, MA, USA; Dermot F.Reilly PhD, Merck Research Laboratories, Genetics and Pharmacogenomics, Boston, Massachusetts, USA; Mathias Gorski, Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany and Department of Nephrology, University Hospital Regensburg, Regensburg, Germany; Vladan Mijatovic on behalf of the CKDGen
consortium, Department of Life and Reproduction Sciences, University of Verona, Verona, Italy; Patricia B. Munroe
PhD, Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London
School of Medicine and Dentistry, Queen Mary University of London, London, UK and NIHR Barts Cardiovascular
Biomedical Research Unit, Queen Mary University of London, London, UK; Georg B. Ehret MD on behalf of the
International Consortium for Blood Pressure; Center for Complex Disease Genomics, McKusick-Nathans Institute
of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA and Cardiology,
Department of Medicine, Geneva University Hospital, Geneva, Switzerland and Institute of Social and Preventive
Medicine (IUMSP), Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland
Alex Thompson PhD, Strategic Epidemiology NewMedicines, UCB, Brussels, Belgium; Maria Uria-Nickelsen PhD,
Clinical Research, Pfizer Worldwide R&D, Cambridge, Massachusetts, USA; Anders Malarstig PhD, Clinical
Research, Pfizer Worldwide R&D, Sollentuna, Sweden; Abbas Dehghan MD PhD on behalf of the CHARGE
inflammation working group, Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The
Netherlands; Thomas F. Vogt PhD, Merck Research Laboratories, Cardiometabolic Disease, Kenilworth, New
Jersey, USA and CHDI Management/CHDI Foundation, Princeton, New Jersey, USA; Taishi Sasaoka MD, PhD,
Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Meidcal and Dental University (TMDU),
Tokyo, Japan; Fumihiko Takeuchi PhD, Norihiro Kato MD, DPhil, Department of Gene Diagnostics and
Therapeutics, Research Institute, National Center for Global Health and Medicine, Japan; Yoshiji Yamada MD,
PhD, Department of Human Functional Genomics, Life Science Research Center, Mie University, Japan; Frank Kee
MD, Director, UKCRC Centre of Excellence for Public Health, Queens, University, Belfast, Ireland; Martina
Müller-Nurasyid PhD, Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center
for Environmental Health, Neuherberg, Germany and Institute of Medical Informatics, Biometry and Epidemiology,
Chair of Genetic Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany and DZHK (German Centre
for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany and Department of Medicine I,
Ludwig-Maximilians-University Munich, Munich, Germany; Jean Ferrières MD, Department of Epidemiology,
UMR 1027- INSERM, Toulouse University-CHU Toulouse, Toulouse, France; Dominique Arveiler MD,
Department of Epidemiology and Public Health, EA 3430, University of Strasbourg and Strasbourg University
Hospital, Strasbourg, France; Philippe Amouyel MD, Department of Epidemiology and Public Health, Institut
Pasteur de Lille, Lille, France; Veikko Salomaa MD, PhD, THL-National Institute for Health and Welfare, Helsinki,
Finland; Eric Boerwinkle PhD, Human Genetics Center, University of Texas Health Science Center at Houston, TX, USA; Simon G. Thompson FMedSci, Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; Ian Ford PhD, University of Glasgow, Glasgow, UK; J. Wouter Jukema MD, Leiden University Medical Center, Leiden, Netherlands; Naveed Sattar MD, Chris J. Packard PhD, University of Glasgow, Glasgow, UK; Abdulla al Shafi Majumder MD, National Institute of Cardiovascular Diseases, Sher-e-Bangla Nagar, Dhaka, Bangladesh; Dewan S Alam MD, PhD, Centre for Global Health Research, St. Michael Hospital, Toronto, ON, Canada; Panos Deloukas PhD, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; Heribert Schunkert MD, Deutsches Herzzentrum München, Technische Universität München, and German Centre for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, all Munich, Germany; Nilesh J. Samani FMedSci, Department of Cardiovascular Sciences, University of Leicester and National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Leicester, UK; Sekar Kathiresan MD on behalf of the MICAD Exome consortium, Broad Institute, Cambridge and Massachusetts General Hospital, Boston, MA, USA; Børge G. Nordestgaard MD, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark; Danish Saleheen* MD, University of Pennsylvania, Philadelphia, US and Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; Joanna M.M. Howson* PhD, Emanuele Di Angelantonio* MD, Adam S. Butterworth* PhD, Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; John Danesh* FMedSci on behalf of the EPIC-CVD Consortium and the CHD Exome+ Consortium, Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK and Wellcome Trust Sanger Institute, Hinxton, UK.
### Table 1: Definitions and source of contributing data for the main study outcome

<table>
<thead>
<tr>
<th>Lp-PLA2 assessment tool</th>
<th>Val279Phe, Loss-of-function variant common in East Asians†</th>
<th>Four loss-of-function variants*, rare in Europeans &amp; South Asians‡</th>
<th>Val379Ala, modest impact variant</th>
<th>Darapladib</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Data sources</strong></td>
<td>Systematic review: Study-level data from up to 12 East Asian studies</td>
<td>De-novo genotyping and participant-level data: up to 8 European or South Asian ancestry studies from the CHD Exome+ Consortium18-25; De-novo genotyping and study-level data: up to 15 European ancestry studies from the CARDIoGRAM consortium1,2; and 3 European ancestry studies from the MICAD Exome consortium28,29. Plus publicly available consortium data</td>
<td>Systematic review: Study-level data from up to 5 randomized clinical trials5,6,38-40 from a systematic review</td>
<td></td>
</tr>
<tr>
<td><strong>Endpoint</strong></td>
<td><strong>Number of studies and unique individuals contributing to analyses; n total or cases / controls</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>7 East Asian studies</td>
<td>8 European or South Asian ancestry studies from the CHD Exome+ Consortium18-25; 15 European ancestry studies from the CARDIoGRAM consortium1; 8 European ancestry studies from the MICAD Exome consortium28,29; 14 European ancestry studies from the C4D consortium30; and 4 European or South Asian ancestry studies from the CARDIoGRAM consortium27.</td>
<td>2 phase III randomized clinical trials of darapladib1-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of cases</td>
<td>Number of controls</td>
<td>Number of cases</td>
<td>Number of controls</td>
</tr>
<tr>
<td></td>
<td>10,088</td>
<td>15,199</td>
<td>35,829</td>
<td>44,948</td>
</tr>
<tr>
<td></td>
<td>32,196</td>
<td>41,464</td>
<td>32,084</td>
<td>58,419</td>
</tr>
<tr>
<td>Lp-PLA2 activity†</td>
<td>12 East Asian studies</td>
<td>1 European ancestry study from the CHD Exome+ Consortium28,29; 1 European ancestry study from the CHARGE Consortium30; 2 European ancestry studies from the CHD Exome+ Consortium2; 3 European ancestry studies from the CARDIoGRAM consortium1; and 3 European ancestry studies from the CHARGE Consortium16.</td>
<td>3 phase II randomized clinical trials38-40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of cases</td>
<td>Number of controls</td>
<td>Number of cases</td>
<td>Number of controls</td>
</tr>
<tr>
<td></td>
<td>8468</td>
<td>1240</td>
<td>8564</td>
<td>2173</td>
</tr>
<tr>
<td>Conventional risk factors§</td>
<td>12 East Asian studies</td>
<td>8 European or South Asian ancestry studies from the CHD Exome+ Consortium18-25; 8 European or South Asian ancestry studies from the CHD Exome+ Consortium18-25; Publicly available consortium data</td>
<td>5 randomized clinical trials5,6,38-40</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>17,898</td>
<td>76,584</td>
<td>51,201</td>
<td>126,142 from 46 studies from the GIANT Consortium41; NA</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td>6705</td>
<td>72,450</td>
<td>71,256</td>
<td>69,245 from 29 studies from the ICBP Consortium42; 323</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td>17,643</td>
<td>76,826</td>
<td>55,431</td>
<td>94,311 from 46 studies from the GLGC Consortium43; 803</td>
</tr>
<tr>
<td><strong>C-reactive protein</strong></td>
<td>2914</td>
<td>40,484</td>
<td>41,442</td>
<td>66,185 from 15 studies from the CHARGE Consortium44; 848</td>
</tr>
<tr>
<td><strong>Glycaemic traits</strong></td>
<td>2914</td>
<td>9420</td>
<td>9408</td>
<td>46,186 from 21 studies from the MAGIC Consortium45; NA</td>
</tr>
<tr>
<td><strong>eGFR</strong></td>
<td>4017</td>
<td>32,929</td>
<td>32,190</td>
<td>74,354 from 26 studies from the CKDGen Consortium46; NA</td>
</tr>
</tbody>
</table>

* In genetic analysis, CHD was defined as myocardial infarction and other major coronary events (~90% of cases) or angiographic stenosis only (~10% of cases); see eTables 2 & 3 for details. In the darapladib analysis CHD was defined as fatal coronary disease, non-fatal MI or urgent revascularization for myocardial ischaemia.

† See eTables 2 & 3 for details on risk factor measurements.

‡ In genetic analysis, CHD was defined as myocardial infarction and other major coronary events (~90% of cases) or angiographic stenosis only (~10% of cases); see eTables 2 & 3 for details. In the darapladib analysis CHD was defined as fatal coronary disease, non-fatal MI or urgent revascularization for myocardial ischaemia.

§ See eTables 2 & 3 for details on risk factor measurements.

¶ In genetic analysis, CHD was defined as myocardial infarction and other major coronary events (~90% of cases) or angiographic stenosis only (~10% of cases); see eTables 2 & 3 for details. In the darapladib analysis CHD was defined as fatal coronary disease, non-fatal MI or urgent revascularization for myocardial ischaemia.

## TABLES

82
Table 2: Comparison on a common scale of human genetic and randomized trial evidence for Lp-PLA₂ lowering and CHD

<table>
<thead>
<tr>
<th></th>
<th>CHD patients</th>
<th>Controls</th>
<th>Risk ratio for CHD per 65% lower Lp-PLA₂ activity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically lowered Lp-PLA₂:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val279Phe (East Asian LoF variant)</td>
<td>10,088</td>
<td>15,199</td>
<td>0.95 (0.88 - 1.03)</td>
</tr>
<tr>
<td>Four LoF variants*</td>
<td>71,362</td>
<td>109,078</td>
<td>0.92 (0.74 – 1.16)</td>
</tr>
<tr>
<td>Val379Ala</td>
<td>82,907</td>
<td>147,029</td>
<td>1.01 (0.68 – 1.51)</td>
</tr>
<tr>
<td>Pharmacologically lowered Lp-PLA₂:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Darapladib</td>
<td>3364</td>
<td>25,490</td>
<td>0.95 (0.89 – 1.02)</td>
</tr>
</tbody>
</table>

* Carriage of any of the four loss-of-function variants c.109+2T>C, Arg82His; Val279Phe; Gln287Ter; LoF = Loss-of-function
Figure 1: Summary of study design

A) Flow chart of study design

B) Exonic structure of the PLA2G7 gene and location of variants used in this study.

UniProt/Swissprot database
1000 Genomes project
Exome sequencing project
ExAc consortium

Genetic inhibition of Lp-PLA₂
- Four loss-of-function variants
- One missense variant

Results from de-novo genotyping, global consortia and systematic literature reviews

Soluble Lp-PLA₂ activity

Conventional cardiovascular risk factors (e.g., lipids)

Coronary disease events

Pharmacological inhibition of Lp-PLA₂
- Darapladib (160mg)

Systematic review of RCTs

PLA2G7 gene coding exons

1 2 3 4 5 6 7 8 9 10 11

rs144983904 Arg82His
rs76863441 Val279Phe
rs140020965 Gln287Ter
rs1051931 Val379Ala

Loss-of-function variants

Modest impact variant

A) Flow chart of study design
B) Exonic structure of the PLA2G7 gene and location of variants used in this study.

ExAc = Exome Aggregation consortium, Lp-PLA₂ = Lipoprotein-associated phospholipase A₂, RCT = Randomized controlled trial, UniProt/Swissprot = Manually annotated and reviewed section of the Universal Protein resource database.
To enable comparison of the magnitude of associations across several different markers, analyses were undertaken with standardized units of measurement for each marker. Associations are presented as per allele change in the biomarker expressed as standard deviations. * Carriage of any of the four loss-of-function variants c.109+2T>C, Arg82His; Val279Phe; Gln287Ter; BMI = Body-mass index, DBP = Diastolic blood pressure, eGFR = estimated glomerular filtration rate, HDL-c = High-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, LoF = Loss-of-function, Lp-PLA2 = Lipoprotein-associated phospholipase A2, SBP = systolic blood pressure. Numbers of participants are provided in Table 1. Details of contributing studies are provided in eTables 2-3.
Figure 3

Spectrum of functional alleles in PLA2G7 and effects on Lp-PLA2 activity (red estimates) and coronary heart disease risk (black estimates); * Carriage of any of the four loss-of-function variants c.109+2T>C, Arg82His; Val279Phe; Gln287Ter; † One study did not provide tabular data to enable calculation of CHD odds ratios in heterozygotes or homozygotes. Hence, numbers are less than those presented for the per allele analysis in Table 2; LoF = Loss-of-function.


cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. Eur J Epidemiol 2007;22(2):129-141.


Harst P, Martin NG, Medland SE, Montgomery GW, Yang J, Chasman DI, Ridker PM, Rose LM, Lehtimaki T,
Jolley J, Sager H, Bornstein SR, Schwarz PE, Kristiansson K, Perola M, Lindstrom J, Swift AJ, Uusitupa M, Atalay M,
Langenberg C, Luan J, Ong KK, Chines PS, Keinanen-Kiukaanniemi SM, Saaristo TE, Edkins S, Franks PW, Hallmans
A, Boehm BO, Kleber ME, Marz W, Winkelmann BR, Kuusisto J, Laakso M, Arveiler D, Cesana G, Kuulasmaa K,
Virtamo J, Yarnell JW, Kuh D, Wong A, Lind L, de FU, Gigante B, Magnusson PK, Pedersen NL, Dedoussis G,
Dimitriou M, Kolovou G, Kanoni S, Stirrups K, Bynncastle LL, Njolstad I, Wilsgaard T, Ganna A, Renberg E,
Hingorani A, Kivimaki M, Kumari M, Assimes TL, Barroso I, Boehnke M, Borecki IB, Deloukas P, Fox CS, Frayling T,
Groop LC, Haritunians T, Hunter D, Ingelsson E, Kaplan R, Mohlke KL, O'Connell JR, Schlessinger D, Strachan DP,
Stefansson K. Sex-stratified genome-wide association studies including 270,000 individuals show sexual

42. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC,
Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go
NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgah M, Palmen J, Vitart V, Braund PS, Kuzeatsova T, Uiterwaal CS,
Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M,
Chang YP, O'Connell JR, Steinle NI, Grobbe DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S,
R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kahonen M, Vilikki J, Adair LS, Lee NR, Chen MH,
Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Moosier V, Chatrurvedi N, Frayling TM, Islam M, Jafar TH,
Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS,
A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T,
Luikkainen LP, Soininen P, Tikkanen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P,
Lyytikainen LP, Soininen P, Tukiainen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P,
Lyytikainen LP, Soininen P, Tukiainen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P,
AC, Tanaka T, Thorand B, Tichet J, Tonjes A, Tuomila T, Uitterlinden AG, van Dijk KW, van HM, Varma D, Visvikis- 
Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhao JH, Zillikens MC, 
Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Rios M, 
Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP. New genetic loci implicated in fasting glucose 

AV, O’Connell JR, Struchalin M, Tanaka T, Li G, Johnson AD, Gierman HJ, Feitosa M, Hwang SJ, Atkinson EJ, 
Demirkan A, Oostra BA, de AM, Turner ST, Ding J, Andrews JS, Freedman B, Koenig W, Illig T, Doring A, 
Wichmann HE, Kolcic I, Zemunik T, Boban M, Minelli C, Wheeler HE, IgI W, Zaboli G, Wild SH, Wright AF, 
Aulchenko YS, Polasek O, Hastie N, Vitart V, Helmer C, Wang JJ, Ruggiero D, Bergmann S, Kahonen M, Viikari J, 
Kronenberg F, Toniolo D, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Siscovick DS, van Duijn CM, 
WH, Fox CS. Genome-wide association and functional follow-up reveals new loci for kidney function. PLoS Genet 