Genetic invalidation of Lp-PLA2 as a therapeutic target: Large-scale study of five functional Lp-PLA2-lowering alleles.

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Genetic invalidation of Lp-PLA₂ as a therapeutic target:

lessons for future cardiovascular trials

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Supplement (comprising a supplementary note and 3 tables, 4 figures)

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ABSTRACT

Background: Darapladib, a potent inhibitor of lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}), has failed to prevent cardiovascular disease outcomes in recent randomized trials. The reasons for this failure are not known.

Objectives: To test whether Lp-PLA\textsubscript{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\textsubscript{2}, known or anticipated to produce a wide range of reductions in soluble Lp-PLA\textsubscript{2} activity. We supplemented de novo genotyping with information on a further 45,823 CHD cases 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\textsubscript{2} activity, conventional cardiovascular risk factors, and CHD risk with corresponding effects of Lp-PLA\textsubscript{2}-lowering alleles.

Results: Compared with non-carriers, Lp-PLA\textsubscript{2} activity was decreased by 94\% (p<10\textsuperscript{-300}) in 279Phe homozygotes. Lp-PLA\textsubscript{2} activity was decreased by 64\% (p=2.4\times10\textsuperscript{-25}) with carriage of any of the four loss-of-function variants, by 45\% (p<10\textsuperscript{-300}) for every allele inherited at Val279Phe, and by 2.7\% (p=1.9\times10\textsuperscript{-12}) for every allele inherited at Val379Ala. Darapladib 160mg once-daily reduced Lp-PLA\textsubscript{2} activity by 65\% (p<10\textsuperscript{-300}). Causal risk ratios for CHD per 65\% lower Lp-PLA\textsubscript{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\textsubscript{2}-lowering alleles was related to CHD risk, suggesting that Lp-PLA\textsubscript{2} is not a valid therapeutic target and that the failure of darapladib could have been anticipated by earlier emergence of human genetic data.

KEY WORDS: Human genetics, target validation, coronary heart disease, Lipoprotein-associated phospholipase A\textsubscript{2}, darapladib
ABBREVIATIONS

CHD = Coronary Heart Disease
CI = Confidence Interval
HDL = High-density lipoprotein
LDL = Low-density lipoprotein
Lp-PLA$_2$ = Lipoprotein-associated phospholipase A$_2$
MI = Myocardial infarction
SD = Standard deviation
INTRODUCTION

Lipoprotein-associated phospholipase A\(_2\) (Lp-PLA\(_2\)), an enzyme expressed by inflammatory cells in atherosclerotic plaques, is carried in the circulation bound predominantly to low-density-lipoprotein (LDL)\(^{(1)}\). Lp-PLA\(_2\) (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-PLA\(_2\) 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-PLA\(_2\) 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-PLA\(_2\) function (i.e., previous studies have been able to consider only one loss-of-function variant in \(PLA2G7\), the gene encoding Lp-PLA\(_2\)).

However, phase 3 randomized trials of darapladib, a potent inhibitor of Lp-PLA\(_2\) activity, have failed to show reductions in cardiovascular risk\(^{(5, 6)}\). This failure could, at least in part, have been due to some 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-PLA\(_2\) inhibition. Trials used statins as background therapy, so Lp-PLA\(_2\) inhibition achieved with statins could have reduced any incremental benefits of darapladib. Trials could not assess the effects of prolonged Lp-PLA\(_2\) inhibition because they recorded only about 3-4 years of median follow-up\(^{(5, 6)}\).

An alternative explanation is that phase 3 trials of darapladib failed because Lp-PLA\(_2\) is not a causal risk factor in cardiovascular disease. We aimed to test this possibility in a large-scale study of CHD by investigating natural loss of Lp-PLA\(_2\) activity acquired through inheritance of any of several Lp-PLA\(_2\)-lowering alleles. Studies of Lp-PLA\(_2\)-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-PLA\(_2\)-lowering alleles should produce lifelong, rather than shorter-term, Lp-PLA\(_2\) 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-PLA\(_2\)-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 database(7), the Exome Aggregation Consortium database (Cambridge, MA, USA; URL: http://exac.broadinstitute.org; [accessed November 2014]), studies of site-directed mutagenesis(8-10) and results from targeted gene sequencing(11). Among the full set of variants identified (eTable1), we selected the following variants that could be detected in the 1000 Genomes(12) or the Exome sequencing(13) 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 documented(14).

Additionally, we studied Val379Ala (rs1051931), a functional variant common in European ancestry populations, which lowers Lp-PLA₂ activity only modestly(10, 15), in contrast with the substantial Lp-PLA₂-lowering achieved by the loss-of-function variants described above.
**Samples and data**

In up to 13,835 participants, we confirmed the associations of functional variants in *PLA2G7* with Lp-PLA₂ activity, using published data from the Atherosclerosis Risk in Communities (ARIC) Study (16), Cardiovascular Health Study (15), Framingham Heart Study (15), Rotterdam study (15), data from de-novo genotyping from the MONICA Risk, Genetics, Archiving, and Monograph (MORGAM) study (17, 18) and Pravastatin in elderly individuals at risk of vascular disease (PROSPER) trial (19), and 12 East Asian studies identified through a systematic review (Table 1, Supplement & eFigure1, eTables 2-3).

In up to 177,343 participants, we quantified associations of functional variants in *PLA2G7* with 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. We used data from our de-novo genotyping which we supplemented with data from existing global genetics consortia (Table 1, eTables 2-3).

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 customised Exome arrays (Illumina, California, USA) by technicians masked to the phenotypic status of the participants’ samples. For the 8 studies of the CHD Exome+ consortium, we had access to participant-level data: the Bangladesh Risk of Acute Vascular Events Study (BRAVE)(20), Copenhagen City Heart Study (CCHS)(21), Copenhagen Ischemic Heart Disease/Copenhagen General Population Study (CIHDS/CGPS)(21), European Prospective Investigation into Cancer and Nutrition-Cardiovascular Disease Study (EPIC-CVD)(22), MORGAM(17, 18), Pakistan Risk of Myocardial Infarction Study (PROMIS)(23), PROSPER(19) and the West of Scotland Coronary Prevention Study (WOSCOPS)(24).

For the 15 studies of the MICAD consortium (which had used similar genotyping methods to those described above but did not genotype c.109+2T>C), we had access to study-level data. 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 3 and Supplement). 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) and Coronary Artery Disease Genetics (C4D) consortia (Table...
1. 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 2-3).

**Randomized trials of darapladib**

To compare genetic associations with effects of pharmacological Lp-PLA\(_2\) inhibition, we conducted a systematic review to identify randomized placebo-controlled trials of darapladib that had reported on Lp-PLA\(_2\) 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 (Supplement).

**Statistical methods**

We defined effect alleles as those associated with lower Lp-PLA\(_2\) activity and assumed an additive model. For participant-level data, we assessed associations of Lp-PLA\(_2\)-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 inheritance(25) to account for population stratification and relatedness among participants. 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 errors 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-PLA\(_2\)-lowering alleles with soluble Lp-PLA\(_2\) 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 principal components. We calculated risk ratios for CHD with decrements in Lp-PLA\(_2\) activity, dividing the log transformed risk ratio and confidence interval (CI) by the effect on Lp-PLA\(_2\) activity of the instrument (ie, the genetic variant)(26). 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 PLA2G7 that lower Lp-PLA\(_2\) 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-PLA\(_2\) activity**

Compared with non-carriers, homozygote carriers of the 279Phe allele had 94% lower Lp-PLA\(_2\) activity (p<10\(^{-300}\)). For each 279Phe allele inherited, Lp-PLA\(_2\) 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-PLA\(_2\) loss-of-function alleles, Lp-PLA\(_2\) activity decreased by 64% (2.25 SD, 2.68-1.83; p=1.6×10\(^{-25}\)). For each 379Ala allele inherited, Lp-PLA\(_2\) 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-PLA\(_2\) 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-PLA\(_2\)–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](#)).
In 2008, GlaxoSmithKline launched a ~$1 billion program of phase 3 trials of darapladib, a compound which has failed in two secondary prevention trials of cardiovascular disease. The results of the current study provide strong evidence that darapladib failed principally because Lp-PLA₂ is not a valid therapeutic target. In retrospect, this failure could, in principle, have been anticipated by earlier emergence of appropriate human genetic data, although it is important to acknowledge that the relevant genetic tools and information used in the current analysis were not available at the time the darapladib program was launched.

To test whether Lp-PLA₂ enzyme activity is causally relevant to CHD, we studied a series of five functional alleles that produced widely differing degrees of reduction in Lp-PLA₂ activity. The extent of reduction in Lp-PLA₂ activity ranged from about 3% for carriers of 379Ala, to about 40% for heterozygote carriers of 279Phe, to about 60% for carriers of any of four loss-of-function variants rare in Europeans and South Asians, to about 95% for homozygote carriers of 279Phe. By comparison, darapladib 160mg once-daily reduced Lp-PLA₂ activity by about 65% in randomized trials. However, across this series of alleles, none of the Lp-PLA₂–lowering alleles we studied was related to CHD risk. In contrast with previous genetic analyses of this topic, our study involved a number of features which has enabled robust causal inference.

First, we studied 92,995 total CHD patients and 162,228 controls, including 182,875 participants for whom we generated new data through de-novo genotyping. Because our study involved almost 20 times more CHD patients than the previous largest study of loss-of-function PLA2G7 alleles, it is the first study to involve adequate power to evaluate effect sizes of relevance to phase 3 trials, such as relative risk reductions for CHD of 10% to 20%.

Second, we considered CHD risk in relation to a series of functional alleles that each reduced Lp-PLA₂ function via different molecular mechanisms, i.e., differing amino acid substitutions due to three different coding variants, or protein truncations due to a nonsense mutation or a splice-site mutation. Because we observed null and broadly concordant findings for CHD risk across this series of alleles that each changed the enzyme in a different way, we could confidently conclude there is no material cause-and-effect relationship between Lp-PLA₂ activity and CHD. By contrast, when the initial phase 3 trial of darapladib was launched in 2008, only two of the five alleles we studied (i.e., Val379Ala and Val279Phe) had yet been identified. Data on Val379Ala, a weak effect missense variant, and CHD risk were inconclusive because studies were under-
powered(27). Data on Val279Phe, a loss-of-function variant, and CHD risk were sparse and restricted to East Asian populations.

Third, using a common numerical scale, we compared Lp-PLA₂-lowering achieved by darapladib treatment versus that achieved by carriage of Lp-PLA₂-lowering alleles. Per 65% reduction in Lp-PLA₂ activity, we found very similar risk ratios (and confidence intervals) for CHD with pharmacological inhibition (i.e., 0.95, 0.89-1.02) as with genetic inhibition (i.e., 0.95, 0.88-1.03). These null, precise, and concordant findings provide compelling evidence against causality.

Fourth, our study involved data from a total of 40 studies involving three major ethnic groups. Hence, the generalizability of our conclusions was considerably enhanced.

Our findings have potential implications for drug development. One implication is the growing importance of human genetic studies to provide validation (or invalidation) of novel drug targets(28-30), enabling the focus of resources on the potentially most fruitful hypotheses. GlaxoSmithKline and other pharmaceutical companies have established major academia-pharma initiatives (www.targetvalidation.org) to support just such activities(29, 31). A specific feature of the current analysis is the illustration of the value of studying a series of functional alleles that have differing quantitative effects on a potential therapeutic target.

A further potential implication relates to the usefulness of human genetic evidence when interpreting observational epidemiological data. Because Lp-PLA₂ is physically linked with LDL through apolipoprotein B, it was previously uncertain in epidemiological biomarker studies whether to make statistical attempts to distinguish the effects of Lp-PLA₂ on CHD risk from those of LDL-cholesterol and other proatherogenic lipids. Hence, previous analyses (including our own(2)) attempted to avoid potential "over-adjustment" by placing emphasis on analyses unadjusted for proatherogenic lipids. However, as the data in the current analysis show that functional alleles in PLA2G7 are not causally relevant to proatherogenic lipids, the present study supports the validity of such adjustment (which, when done, substantially attenuate associations between Lp-PLA₂ activity and CHD risk(2)).

Our study had potential limitations. Carriage of 279Phe is known to produce a misfolded version of Lp-PLA₂ not secreted by cells, prompting suggestions that it could produce “off-target” effects such as increased cell death(32, 33). However, our observation of null associations between four other functional alleles in PLA2G7 and CHD, each of which operates via a different molecular mechanism, argues against this explanation. Lifelong genetic reductions in Lp-PLA₂ could result in compensatory responses that increase
cardiovascular risk. However, this explanation seems unlikely because it would require any such compensation to apply similarly across an extremely wide range of genetically-produced reductions in Lp-PLA₂ and, moreover, it could not operate through known cardiovascular mechanisms (because we observed no associations between Lp-PLA₂-lowering alleles and a panel of 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.

In summary, none of a series of Lp-PLA₂-lowering alleles was related to CHD risk, suggesting that Lp-PLA₂ is not a valid therapeutic target.

**CLINICAL PERSPECTIVES**

**Competency in medical knowledge:** Inhibition of Lp-PLA₂ via darapladib failed in reducing recurrent CHD events because Lp-PLA₂ is not a valid therapeutic target.

**Translational Outlook:** Evaluation of drug targets for CHD (and likely other disease areas) using human genetic studies can help shift resources in drug development to more fruitful hypotheses.
**Funding:** 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.
**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.

PLA2G7 gene coding exons

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

**Conventional cardiovascular risk factors (eg, lipids)**

**Soluble Lp-PLA₂ activity**

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

**Coronary disease events**

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

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

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

PLA2G7 gene coding exons

1 2 3 4 5 6 7 8 9 10 11

- rs144983904 Arg82His
- rs76863441 Val279Phe
- rs140020965 Gln287Ter
- rs1051931 Val379Ala

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.
**Figure 2** Mean per allele differences in Lp-PLA<sub>2</sub> activity and cardiovascular risk factor levels by Lp-PLA<sub>2</sub> lowering alleles or with darapladib 160mg daily

To enable comparison of the magnitude of associations across several different markers, analyses were undertaken with standardised 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-PLA<sub>2</sub> = Lipoprotein associated phospholipase A<sub>2</sub>, SBP = systolic blood pressure. Numbers of participants are provided in Table 1. Details of contributing studies are provided in eTables 2-3.
**Figure 3:** Association of Lp-PLA$_2$-lowering alleles with Lp-PLA$_2$ activity and CHD risk

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
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Studies / Consortium</th>
<th>Number of unique individuals contributing to analyses; n total / cases / controls</th>
<th>Assessment method / endpoint definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease</td>
<td>8 European or South Asian ancestry studies with de-novo genotyping and participant level data</td>
<td>35,829 cases / 43,948 cases / 41,166 controls</td>
<td>Myocardial infarction and other major coronary events (~90% of cases); angiographic stenosis only (~10% of cases); eTable 2 and eTable 3 for details</td>
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<tr>
<td></td>
<td>15 European ancestry studies with de-novo genotyping and study-level data from the MICAExome consortium</td>
<td>35,533 cases / 64,130 cases / 32,084 controls</td>
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<tr>
<td></td>
<td>14 European ancestry studies from the CARDIoGRAM consortium(34)</td>
<td>20,315 cases / 58,419 controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 European or South Asian ancestry studies from the C4D consortium(35)</td>
<td>15,420 cases / 15,062 controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 East Asian studies</td>
<td>10,988 cases / 15,199 controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 phase III randomized clinical trials of darapladib(5, 6)</td>
<td>3364 cases / 25,490 non-cases</td>
<td></td>
</tr>
<tr>
<td>Lp-PLA₂ activity</td>
<td>2 European ancestry studies with de-novo genotyping and 4 previously published European ancestry studies (15, 16)</td>
<td>9804 cases / 13,835</td>
<td>Validated colorimetric or radioactive assays (eTable 2 for details)</td>
</tr>
<tr>
<td></td>
<td>12 East Asian studies</td>
<td>8468 cases / 854</td>
<td>Validated colorimetric assay</td>
</tr>
<tr>
<td></td>
<td>3 phase II randomized clinical trials(36-38)</td>
<td>854</td>
<td></td>
</tr>
<tr>
<td>Conventional cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>8 studies with de-novo genotyping and 46 studies from the Genetic Investigation of ANthropometric Traits (GIANT) consortium(39); and 12 East Asian studies</td>
<td>17,898 cases / 76,584 / 177,343 cases / NA</td>
<td>Validated assays – see eTables 2 &amp; 3 for full details of each risk factor and study/consortium</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>8 studies with de-novo genotyping and 29 studies from the International Consortium for Blood Pressure (ICBP)(40); and 12 East Asian studies; and 5 randomized clinical trials(5, 6, 36-38)</td>
<td>6705 cases / 72,450 / 140,901 cases / 323</td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>8 studies with de-novo genotyping and 46 studies from the Global Lipids Genetics Consortium (GLGC)(41); and 12 East Asian studies; and 5 randomized clinical trials(5, 6, 36-38)</td>
<td>17,643 cases / 76,826 / 149,742 cases / 803</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>8 studies with de-novo genotyping and 15 studies from the Cohort for Health and Ageing Research information-walking group (CHARGE)(42); and 12 East Asian studies; and 5 randomized clinical trials(5, 6, 36-38)</td>
<td>2914 cases / 40,484 / 107,627 cases / 848</td>
<td></td>
</tr>
<tr>
<td>Glycaemic traits</td>
<td>8 studies with de-novo genotyping and 21 studies from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC)(43); and 12 East Asian studies</td>
<td>2914 cases / 9420 / 55,594 cases / NA</td>
<td></td>
</tr>
<tr>
<td>eGFR</td>
<td>8 studies with de-novo genotyping and 26 studies from the Chronic Kidney Disease Genetics (CKDGen) consortium(44); and 12 East Asian studies</td>
<td>4017 cases / 32,929 / 106,544 cases / NA</td>
<td></td>
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</tbody>
</table>

*rs142974898 (c.109+2T>C), rs144983904 (Arg82His), rs76863441 (Val279Phe), rs140020965 (Gln287Ter); see also Figure 1B for further variant details; BMI = Body-mass index, eGFR = estimated glomerular filtration rate, NA= Data not available. Further detail on the individual studies is provided in eTables 2-3
Table 2: Comparison on a common scale of human genetic and randomized trial evidence for Lp-PLA$_2$ lowering and CHD

<table>
<thead>
<tr>
<th></th>
<th>CHD patients</th>
<th>Controls</th>
<th>Risk ratio for CHD per 65% lower Lp-PLA$_2$ activity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetically lowered Lp-PLA$_2$</strong></td>
<td></td>
<td></td>
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<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>
</tbody>
</table>

| **Pharmacologically lowered Lp-PLA$_2$** |          |          |                                                               |
| Darapladib               | 3364      | 25,490   | 0.95 (0.89 – 1.02)                                            |

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


with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. J Am Coll Cardiol 2008; 51:1632-41.


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