A replicated, genome-wide significant association of aortic stenosis with a genetic variant for Lp(a): meta-analysis of published and novel data

SHORT TITLE: Genetic variants for Lp(a) and aortic stenosis

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Trials of dedicated Lp(a) lipoprotein-lowering agents[1] have refocused interest on Lp(a) as a causal risk factor for coronary artery disease (CAD), and potentially for aortic valve stenosis (AS). Elevated plasma Lp(a) concentrations have been linked to causal variants for CAD (chiefly rs10455872 and rs3798220) at the *LPA* locus on chromosome 6q26-27.[2] The rs10455872 variant is also the sole genome-wide significant variant associated with aortic valve calcium.[3] Several small studies have tentatively replicated this association, not only for aortic valve calcium, which does not necessarily cause functional impairment of the valve, but also for clinically relevant AS.[3-5] However, none of these studies has definitively established that rs10455872 is associated with clinically relevant AS. We conducted a meta-analysis of associations of rs10455872 and rs3798220 variants with AS using evidence from all published studies, together with novel data from the UK Biobank.

Approximately 152,000 UK Biobank participants had rs10455872 and rs3798220 assayed using Affymetrix Axiom arrays (see http://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=155580). All participants gave written informed consent to a protocol approved by the North West Multicentre Research Ethics Committee (UK). Genotype data were available for 112,186 eligible participants after exclusions for genomic quality control, genotype missingness, gender mismatch, relatedness, and non-British Caucasian ancestry. Minor allele frequencies were 0.08 for rs10455872 (G allele) and 0.02 for rs3798220 (C allele), there was no evidence of deviations from Hardy-Weinberg equilibrium, and the variants were independent (linkage disequilibrium r^2=0.0015). We identified 535 cases of non-rheumatic AS (ICD-10: I35.0 and I35.2) from hospital and death records, and matched each case to up to 20 controls, by sex, age, and recruitment centre (total: 10,617 controls). Among the 535 AS cases, 259 (48%) had aortic valve

replacement at or after AS diagnosis, and 263 (49%) had concomitant CAD, whereas proportionally fewer controls (1434/10617, 14%) had any evidence of CAD. Odds ratios were estimated by logistic regression, adjusting for sex, age, centre, genotyping batch, and the first 10 principal components of genotype.

Searching EMBASE and MEDLINE (Ovid and PubMed) and cited reference lists, we identified published studies of *LPA* variants and AS: the Malmö Diet and Cancer Study (MDCS);[3] combined analysis of the Copenhagen City Heart Study (CCHS) and Copenhagen General Population Survey (CGPS);[4] and, separately, the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk, and the Montreal Heart Institute (MHI) Biobank.[5] In the MHI Biobank, cases were ascertained by echocardiography, whereas all other studies used coded electronic health records. Published estimates were converted to per-allele relative risks (RR) by weighted regression. Under the rare disease assumption, odds ratios were treated as relative risks. All studies reported estimates for rs10455872.[3-5] Only CCHS and CGPS reported an estimate for rs3798220.[4] Estimates were combined by fixed effect meta-analysis.

Results are shown in the **Figure**. Combining previous studies only, AS was significantly associated with rs10455872 at the genome-wide level (RR=1.65, 95%CI 1.43-1.90; $P=4\times10^{-12}$; $I^2=0\%$ for heterogeneity). In CCHS and CGPS, AS was not associated with rs3798220. In UK Biobank, AS was associated both with rs10455872 (RR=1.69, 95%CI 1.39-2.05; $P=1\times10^{-7}$), and with rs3798220 (RR=1.63, 95%CI 1.11-2.38; $P=1\times10^{-2}$). Combined analysis of previous studies with UK Biobank gave summary RRs of 1.66 (95%CI 1.48-1.86; $P=2\times10^{-18}$; $I^2=0\%$) for rs10455872 and 1.43 (95%CI 1.04-1.99; $P=3\times10^{-2}$; $I^2=39\%$) for rs3798220. A random effect model for rs3798220 gave RR=1.37 (95%CI 0.87-2.16; $P=2\times10^{-1}$).

Plasma Lp(a) is a recognised risk factor for AS, but rs10455872 is the first genetic variant affecting Lp(a) levels to be robustly linked to the disease, with genome-wide significance in published studies and replication in UK Biobank. The association of rs10455872 with AS was highly consistent between studies, although estimates of absolute differences in plasma Lp(a) by rs10455872 genotype have varied between approximately 20 and 50 mg/dL per minor allele.[3-5] Together with our meta-analysis, these estimates suggest a 10-30% greater risk of AS per 10 mg/dL greater genetically-determined Lp(a) level. However, absolute risks of AS associated with particular Lp(a) concentrations have yet to be elucidated, particularly in the context of concomitant CAD. The relative risk for AS per minor allele of rs10455872 was similar to that for CAD,[2, 3] consistent with an independent effect, but further studies are required to clarify the relationships between CAD and AS. AS risk may also be related to rs3798220, but there was limited statistical power for this low frequency variant.

The strong and consistent associations of rs10455872 with AS risk confirm a probable causal role of high plasma Lp(a) in clinically relevant AS, additional to its previously reported association with aortic valve calcium. This study also provides strong support for Lp(a) as a therapeutic target for AS prevention. Novel Lp(a)-lowering agents can substantially reduce Lp(a) levels,[1] and further development of these agents will be aided by better quantification of the relationships between *LPA* variants, plasma Lp(a) levels and AS risk. Continued genetic discovery may reveal additional therapeutic targets to prevent or slow progression of aortic stenosis.

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Author contributions

All authors contributed to the design of the study. BC analysed the data and wrote the first draft of the manuscript. All authors contributed to interpreting the data, and to critically revising the manuscript for intellectual content.

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Conflicts of interest

Dr Thanassoulis has participated in Advisory Boards for Amgen and Ionis Pharmaceuticals, and is a consultant to Ionis Pharmaceuticals. The other authors report no conflicts of interest.

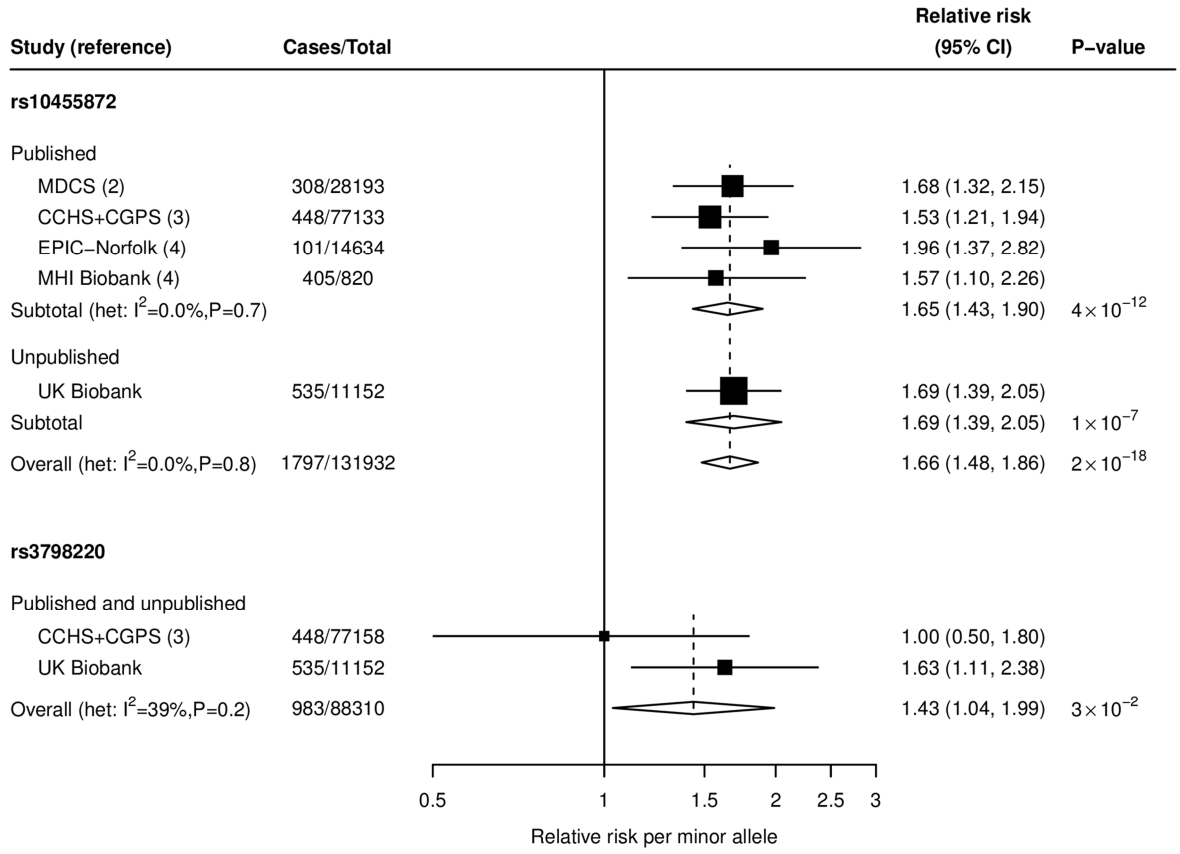


Figure. Meta-analysis of aortic stenosis risk in relation to *LPA* variants rs10455872 and rs3798220

Each individual study estimate is represented as a square, with area proportional to the inverse of its variance, which is its weight in the fixed effect meta-analysis; summary estimates are shown as diamonds. Abbreviations: CI: confidence interval; het: heterogeneity; for study abbreviations, see text.