SUPPLEMENTARY DATA FILE

Lipoprotein-Associated Phospholipase A2 activity, Genetics, and Calcific Aortic Valve Stenosis in Humans

Running title: Lp-PLA2 and calcific aortic valve stenosis

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Supplementary Methods

QUEBEC-CAVS

Patients with severe CAVS undergoing aortic valve replacement (AVR) were recruited at the *Institut universitaire de cardiologie et de pneumologie de Québec* (IUCPQ). Only cases with tricuspid nonrheumatic CAVS were included. No severe regurgitation or other severe valvular heart diseases were present. In parallel, a control group was recruited from patients that underwent cardiac surgery, mostly for isolated coronary artery bypass (>98%). Other indications for surgery in the control group included heart transplant, tumor removal, aortic endoprosthesis, and interatrial communication. Absence of CAVS was confirmed by echocardiography. Patients with a history of severe valvular heart disease (at any of the four valves), with significant aortic valve regurgitation (grade > 2/4) or with end-stage renal disease (estimated glomerular filtration rate < 15 mL/min/1.73 m^2) were excluded. Patients with CAVS and controls were free of congenital heart defects. All patients signed an informed consent including for genetic studies. The study was approved by the ethics committee of the IUCPQ. Demographics, anthropometric measurements, lifestyle factors, previous and current medical history, current medication, and blood pressure measurements were collected and have previously been published.[1] The analysis included 1009 cases and 1017 controls.

UK Biobank

UK Biobank is a large prospective cohort of about 500,000 individuals between 40 and 69 years old recruited from 2006 to 2010 in several centers located in the United Kingdom.[2] The present analyses were conducted under UK Biobank data application number 25205. We used genotyping data obtained from the second genetic data release, including 488,377 individuals. Samples were genotyped with the Affymetrix UK BiLEVE Axiom array or the Affymetrix UK Biobank Axiom Array. Phasing and imputation were performed centrally using a reference panel combining the Haplotype Reference Consortium (HRC) as a first choice and UK10k and 1000 Genomes Phase 3 samples for SNPs not available in HRC. Samples with call rate <95%, outlier heterozygosity rate, gender mismatch, non-white British ancestry, related samples (second degree or closer), samples with excess third-degree relatives (> 10), or not used for relatedness calculation were excluded. CAVS diagnosis was established from hospital records, using the International Classification of Diseases version-10 (ICD10) and Office of Population Censuses and Surveys Classification of Interventions and Procedures (OPCS-4) coding. CAVS was defined as ICD10 code number I35.0 or I35.2. Participants with a history of rheumatic fever or rheumatic heart disease as determined by ICD10 codes I00–I02 and I05–I09 were excluded from the CAVS group. We included all other participants in the control group, except for those with OPCS-4 codes K26 or K30.2 or a self-reported diagnosis of CAVS, which were excluded from the analysis.

EPIC-Norfolk

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk prospective population study is a population-based cohort of 25,639 men and women aged between 39 and 79 years residing in Norfolk, United Kingdom. The design and methods of the study have been described in details.[3, 4] Participants were recruited from age–sex registers of general practices in

Norfolk as part of the 10-country collaborative EPIC study. At the baseline survey conducted between 1993 and 1997, participants completed a detailed health-and-lifestyle questionnaire. Hospitalizations of study participants were identified through the East Norfolk Health Authority database, which records all hospital contacts throughout England and Wales for Norfolk residents. Vital status for all EPIC-Norfolk participants was obtained through death certification at the Office for National Statistics. The underlying cause of death or hospital admission was coded by trained nosologists according to the ICD10. Participants were identified as having incident aortic stenosis if they were hospitalized with AVS as an underlying cause or if they died with AVS as an underlying cause. The Norwich District Health Authority Ethics Committee approved the study, and all participants gave signed informed consent.

Genetic Epidemiology Research on Aging (GERA)

The GERA cohort is a population-based cohort of more than 100,000 adults living in Northern California. All participants are members of the Kaiser Permanente Northern California integrated health care delivery system and provided written, informed consent (database of Genotypes and Phenotypes study accession phs000674.v2.p2). The study was approved by the relevant internal review boards at Kaiser Permanente Northern California and the McGill University Health Centre. CAVS cases, determined through extracting electronic health records data from January 1996 to December 2015, inclusive, were defined based on the presence of either: an ICD, Ninth Revision (ICD9) code for CAVS (ICD9 424.1), or a procedure code for a prior AVR. Controls were study participants without an ICD-9 code for CAVS or a procedure code for AVR. This analysis was restricted to participants who self-reported only European descent, as there were insufficient numbers of individuals available of other races/ethnicities.

Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study (MDCS) is a prospective, population-based cohort study from the city of Malmö in southern Sweden. Data collection, sample characteristics, and clinical definitions of prevalent and incident CAVS for MDCS have been described previously.[5]

French Datasets

A large cohort of “isolated” CAVS cases has been constituted by l’institut du thorax in Nantes. Doppler-echocardiography and blood sampling was carried out at the time of enrollment. Patients with severe renal failure, history of rheumatic disease or chest radiation were excluded. A total of 1663 severe CAVS cases were recruited between 2001 to 2017 at Nantes, Rennes and Angers University Hospitals. Most of the patients were referred to surgery after enrollment. The study was approved by the local ethics committee and all patients provided informed consent for the purpose of genetic studies. Coronary artery disease was defined as previously described in the QUEBEC-CAVS project. In parallel, the Cardiovascular department in Bichat university Hospital in Paris has recruited 1500 patients (GENERAC and COFRASA projects). Blood samples and valve tissues are collected (DNA, blood and tissue bank stored at the level of the Center of Biological Resources). The control populations came from two datasets called D.E.S.I.R and P.R.E.G.O. (*Population de Référence du Grand Ouest*). D.E.S.I.R. (The Data from the Epidemiological Study on the Insulin Resistance Syndrome) [6] is an Epidemiological cohort which is used here as a control general population. The P.R.E.G.O. is a set of 5707 healthy

persons selected through the Blood Donor Service, originating from Western France, as a resource dedicated to providing a regional reference population of Western France for national and international research projects in the field of evolution, population and medical genetics. CAVS and CAD status was not available in D.E.S.I.R. and P.R.E.G.O. because of the lack of cardiac echo data in this cohort. The patients were genotyped in three waves. In CAVS-France 1, 1329 patients from the *institut du thorax* biobank were genotyped using Axiom Genome-Wide CEU-1 array (Affymetrix, Inc). We used a general population as controls: a subset of 901 individuals from D.E.S.I.R. and 466 from P.R.E.G.O. After quality controls (genotyping rate and heterozygosity) and a selection on individuals (relatedness and demographic stratification), we kept 1261 patients (741 tricuspids, 168 bicuspids, 352 ambiguous), 865 individuals from D.E.S.I.R. and 440 from P.R.E.G.O. In CAVS-France 2, study participants were genotyped using Axiom Genome-Wide PMRA array (Affymetrix, Inc). The dataset is composed of a set of 1478 patients recruited at the Hôpital Bichat and 319 patients from the *institut du thorax* biobank and 2828 controls from P.R.E.G.O. In patients, we observed 946 tricuspids, 317 bicuspids and 534 whose status was ambiguous. We made the same quality controls and selection on individuals as cohort CAVS-France 1 and kept at the end 1181 patients from Hôpital Bichat, 314 patients from the *institut du thorax* biobank (807 tricuspids, 254 bicuspids and 434 ambiguous) and 2707 controls. The French CAVS-France 3 dataset is composed of a set of 379 patients from the *institut du thorax* biobank and 2743 controls from P.R.E.G.O. All patients had tricuspid valve. We made the same quality controls and selection on individuals as cohorts CAVS-France 1 and CAVS-France 2 and we kept at the end 367 patients and 2519 controls.

Cohorts for Heart and Aging Research in Genetic Epidemiology consortium

Finally, the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium is an ongoing investigator-driven collaboration among several large, population-based cohort studies in which genome-wide genotype data have been obtained and comprehensive individual phenotyping for a variety of clinical characteristics has been performed. All participants were of white European ancestry and had undergone genotyping and computed tomography (CT) scanning for the presence of aortic valve calcification (n=6940). Details of this study methodology have been described previously [5]. Data for each SNP is available online (https://www.framinghamheartstudy.org/fhs-for-researchers/gwas-study-results, accessed in September 2018).

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Supplementary Table 1: Association of SNPs at the PLA2G7 loci with traits in the PhenoScanner v2

SNP	Trait	Study	PMID	Dataset
rs7756935	Lp-PLA2 activity	Grallert H	22003152	GRASP
rs7756935	Lipoprotein associated phospholipase A2 activity and mass	Grallert H	22003152	NHGRI-EBI_GWAS_Catalog
rs7756935	1 alkyl 2 acetylglycerophosphocholine esterase	Grallert H	22003152	dbGaP
rs1421368	Lp-PLA2 activity	Grallert H	22003152	GRASP
rs1421368	Viral infection of unspecified site	Neale B	UKBB	Neale-B_UKBB_EUR_2017
rs1805017	Lp-PLA2 activity	Grallert H	22003152	GRASP
rs1805017	Lp-PLA2 mass	Suchindran S	20442857	GRASP
rs1805017	Lp-PLA2 mass	Grallert H	22003152	GRASP
rs1805017	Lipoprotein associated phospholipase A2 activity and mass	Suchindran S	20442857	NHGRI-EBI_GWAS_Catalog
rs1805017	Lipoprotein associated phospholipase A2 activity and mass	Grallert H	22003152	NHGRI-EBI_GWAS_Catalog
rs1805017	1 alkyl 2 acetylglycerophosphocholine esterase	Suchindran S	20442857	dbGaP
rs1805017	1 alkyl 2 acetylglycerophosphocholine esterase	Grallert H	22003152	dbGaP
rs4498351	Lp-PLA2 mass	Grallert H	22003152	GRASP