Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Methods

Study populations

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 for the realization of 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 who are 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. Individuals with congenital heart disease (ICD9 746-747) were excluded. 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.

French Datasets

A large cohort of “isolated” CAVS cases has been constituted by l’institut du thorax in Nantes. Doppler-echocardiography and blood sampling were 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) (5) 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 provide 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.

Family study

A series of consecutive patients with mild to severe CAVS who did not undergo AVR were recruited at the echocardiography lab of the IUCPQ. Exclusion criteria included the presence of mitral valve stenosis, mitral insufficiency (>2), aortic insufficiency (>2) and heart failure (ejection fraction <40%). Cases were excluded if they had CAVS with rheumatic etiology or if they had any type of cancer that required radiotherapy in the thoracic area (breast, trachea, bronchus or lung cancer) prior to the diagnostic of CAVS. Women were also excluded if they were pregnant or lactating. Lp(a) levels were measured in patients with CAVS and first-degree relatives of patients with CAVS and Lp(a) levels ≥60 mg/dL (brothers, sisters or children aged above 40) as well as a control group of participants with Lp(a) levels <60 mg/dL unrelated to patients with CAVS. Monthly interrupted time series were performed to recruit individuals without CAVS undergoing Doppler echocardiography (controls with normal aortic valves). Additional controls were also recruited through the echocardiography laboratory and advertisement at the IUCPQ. Additional first-degree relatives of patients who underwent AVR for CAVS and Lp(a) levels ≥60 mg/dL from the IUCPQ tissues biobank were recruited. Each participant completed detailed questionnaires about their health status, medication, cardiovascular history, family history and ethnic origins. Previous and current medical history included history of smoking, documented diagnoses of hypertension [patients receiving antihypertensive medications or having known but untreated hypertension (blood pressure ≥ 140/90 mmHg)], diabetes (fasting glucose ≥ 7 mmol/l or treatment with anti-diabetic medication), and detailed information on current medication was collected. Body weight, height and waist circumference were measured following standardized procedures. Blood pressure and heart rate were also assessed. Peak aortic jet velocity, peak and mean transvalvular gradients aortic valve area were measured and aortic valve morphology

was assessed by Doppler Echocardiography (6,7). The study protocols were approved by the Ethics Committees of the IUCPQ and all patients signed a written informed consent. The definition of CAD included a history of myocardial infarction, angioplasty, coronary artery bypass or angina.

Assessment of aortic valve microcalcification by 18F-NaF PET/CT

We assessed aortic valve micro calcification by 18F-NaF PET/CT in 33 first-degree relatives free of echocardiographic diagnosis of CAVS aged 40 years or older of 17 patients with CAVS and Lp(a) levels ≥60 mg/dLas well as in a control group of 23 participants with Lp(a) levels <60 mg/dLfrom the Quebec cohort. All participants who underwent 18F-NaF PET/CT had plasma creatinine levels >30 μmol/L (assessed <4 weeks before PET/CT). 18F-NaF PET/CT were performed on integrated PET/CT scanners (GE Discovery RX16). Doses of 125 MBq of 18F-NaF were injected intravenously. PET scanning started after a 60-minute uptake period for a 30-minutes bed time. Then two attenuation-correction CT scans (low dose 120 kV, 50 mAs) were performed centered over the aortic valve, one without contrast followed by a second one after injection of 80 and 105 ml of isomolar contrast medium. Analyses of aortic valve calcification and NaF uptake were performed offline on a dedicated platform (OsiriX MD). Aortic valve calcification was measure on the non-contrast ECG-gated breath-hold CT and expressed in arbitrary unit. PET and contrast CT images were re-orientated into the plane of the aortic valve and circular regions of interest (ROIs) were drawn on adjacent 3-mm slices from the ascending aorta to the left ventricular outflow tract to assess the entire valve. The mean and maximum standard uptake values (SUVs) were calculated for each slice, the two highest score from contiguous slices were averaged, and corrected for blood pool activity (right atrium) to provide mean tissue-to-background ratios (TBRs). We selected a threshold of 1.25 to identify individuals with active aortic valve microcalcification. This value represents the mean TBR of participants without CAVS with high Lp(a) levels (≥60 mg/dL) in a pilot investigation that we conducted.

These images were analyzed by an investigator blinded to all clinical, echocardiographic and laboratory data (M.A.C.).

eReferences.

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eTable 1. Characteristics of the selected single nucleotide polymorphism associated with Lp(a) concentrations characteristics.

SNP	Chromosome position (GRCh37)	Modeled allele	Beta	Modeled allele frequency QUEBEC-CAVS	Modeled allele frequency UK Biobank	Modeled allele frequency EPIC-Norfolk	Modeled allele frequency GERA	Modeled allele frequency France 1	Modeled allele frequency France 2	Modeled allele frequency France 3
rs3798220	6:160961137	C	0.98	0.030	0.018	0.017	0.016	0.016	0.014	0.019
rs10455872	6:161010118	G	0.91	0.097	0.081	0.079	0.067	0.071	0.066	0.069
rs41272114	6:161006077	C	0.62	0.980	0.962	-	0.966	0.966	0.956	0.964

eTable 2. Clinical characteristics of the Québec-CAVS cohort.

	CAVS	Controls	p-value	CAVS with CAD	Controls with CAD
N	1009	1017		586	989
Male	642 (63.6)	660 (64.9)	0.55	427 (72.9)[b]	644 (65.1)[a]
Age (Years)	72.5 ± 8.4	70.9 ± 8.1	<0.001	72.8 ± 8.2[b]	71.0 ± 8.0[a]
BMI (kg/cm2)	28.6 ± 5.4	27.7 ± 4.6	<0.001	28.5 ± 5.2[b]	27.7 ± 4.6[a]
CAD	586 (58.1)	989 (97.2)	<0.001		
Type 2 diabetes	327 (32.4)	330 (32.4)	0.98	212 (36.2)	326 (33.0)
Hypertension	763 (75.6)	763 (75.0)	0.76	464 (79.2)	747 (75.5)
Active smokers	87 (8.6)	124 (12.2)	0.0016	53 (9.0)	118 (11.9)
Lipid-lowering therapy	733 (72.6)	895 (88.0)	<0.001	476 (81.2)[b]	875 (88.5)[a]

Data are presented as mean (SD) or n (%)
a: vs CAVS with CAD
b: vs controls with CAD

eTable 3. Clinical characteristics of the UK Biobank cohort.

	CAVS	Controls	p-value	CAVS with CAD	Controls with CAD	CAVS without CAD	Controls without CAD
N	1350	349,043		819	26,112	531	322,931
Male	899 (66.6)	162,125 (46.4)	<0.001	596 (72.8)[c]	18,180 (69.6)[d]	303 (57.1)[a,d]	143,945 (44.6)[b,c]
Age (Years)	62.5 ± 5.8	56.9 ± 7.9	<0.001	62.9 ± 5.3[b]	61.5 ± 6.1[a,d]	61.9 ± 6.4[d]	56.5 ± 7.9[b,c]
BMI (kg/cm2)	29.4 ± 5.3	27.4 ± 4.8	<0.001	29.8 ± 5.2[b,c]	29.2 ± 5.0[a,d]	28.7 ± 5.3[a,d]	27.3 ± 4.7[b,c]
CAD	819 (60.7)	26,112 (7.5)	<0.001				
Type 2 diabetes	272 (20.1)	15,866 (4.5)	<0.001	203 (24.8)[b,c]	4554 (17.4)[a,d]	69 (13.0)[a,d]	11,312 (3.5)[b,c]
Hypertension	1000 (74.1)	111,312 (31.9)	<0.001	656 (80.1)[b,c]	18,435 (70.6)[a,d]	344 (64.8)[a,d]	92,877 (28.8)[b,c]
Active smokers	156 (11.6)	35,206 (10.1)	0.072	104 (12.7)[c]	3574 (13.7)[d]	52 (9.8)[a]	31,632 (9.8)[b]
Lipid-lowering therapy	729 (54.0)	64,464 (18.5)	<0.001	525 (64.1)[c]	16,529 (63.3)[d]	204 (38.4)[a,d]	47,935 (14.8)[b,c]

Data are presented as mean (SD) or n (%)
a: vs CAVS with CAD
b: vs controls with CAD
c: vs CAVS without CAD
d: vs controls without CAD

eTable 4. Clinical characteristics of the EPIC-Norfolk cohort.

	CAVS	Controls	p-value	CAVS with CAD	Controls with CAD	CAVS without CAD	Controls without CAD
N	508	20,421		334	4201	174	1622
Male	286 (56.3)	9438 (46.2)	<0.001	203 (60.8)[c]	2625 (62.5)[d]	83 (47.7)[a]	6813 (42.0)[b]
Age (Years)	64.5 ± 7.6	59.0 ± 9.3	<0.001	64.2 ± 7.4	64.1 ± 8.1[d]	65.0 ± 8.0[d]	57.7 ± 9.1[b,c]
BMI (kg/cm2)	27.4 ± 3.8	26.3 ± 3.8	<0.001	27.5 ± 3.9[b]	27.1 ± 3.8[a,d]	27.0 ± 3.7[d]	26.1 ± 3.8[b,c]
CAD	334 (65.7)	4201 (20.6)	<0.001				
Type 2 diabetes	15 (3.0)	444 (2.2)	0.24	14 (4.2)[c]	234 (5.6)[d]	1 (0.6)[a]	210 (1.3)[b]
Hypertension	136 (26.8)	3052 (14.9)	<0.001	99 (29.6)[c]	1140 (27.1)[d]	37 (21.3)[a,d]	1912 (11.8)[b,c]
Active smokers	51 (10.1)	2331 (11.5)	0.0024	30 (9.1)[c]	554 (13.3)[d]	21 (12.1)[a]	1777 (11.0)[b]
Lipid-lowering therapy	77 (15.2)	1591 (7.8)	<0.001	57 (17.1)[b,c]	535 (12.7)[a,d]	20 (11.5)[a,d]	1056 (6.5)[b,c]

Data are presented as mean (SD) or n (%)
a: vs CAVS with CAD
b: vs controls with CAD
c: vs CAVS without CAD
d: vs controls without CAD

eTable 5. Clinical characteristics of the GERA cohort.

	CAVS	Controls	p-value	CAVS with CAD	Controls with CAD	CAVS without CAD	Controls without CAD
N	3469	51711	<0.001	2105	10787	1364	40924
Age (Years)	74.57 ± 8.49	67.40 ± 8.46	<0.001	75.39 ± 8.25[b,c]	71.53 ± 8.72[a,d]	73.30 ± 8.68[a,d]	66.32 ± 8.05[b,c]
BMI (kg/cm2)	27.44 ± 5.41	26.84 ± 5.06	<0.001	27.73 ± 5.40[c]	27.52 ± 5.07[d]	27.00 ± 5.38[a,d]	26.66 ± 5.04[b,c]
CAD	2105 (60.7)	10787 (20.9)	<0.001				
Type 2 diabetes	608 (17.5)	5351 (10.3)	<0.001	444 (21.1)[b,c]	2017 (18.7)[a,d]	164 (12.0)[a,d]	3334 (8.1)[b,c]
Hypertension	1943 (56.0)	20054 (38.8)	<0.001	1273 (60.5)[b,c]	5661 (52.5)[a,d]	670 (49.1)[a,d]	14393 (35.2)[b,c]
Smokers*	1834 (56.2)	24533 (49.6)	<0.001	1163 (59.2)[c]	5917 (58.3)[d]	671 (51.8)[a,d]	18616 (47.4)[b,c]
Dyslipidemia	2670 (77.0)	28667 (55.4)	<0.001	1847 (87.7)[b,c]	9036 (83.8)[a,d]	823 (60.3)[a,d]	19631 (48.0)[b,c]

Data are presented as mean (SD) or n (%)
*: Smoked at least once during lifetime
a: vs CAVS with CAD
b: vs controls with CAD
c: vs CAVS without CAD
d: vs controls without CAD

eTable 6. Clinical characteristics of the French cohorts.

	CAVS	CAVS with CAD	CAVS without CAD
N	3076	1892	1153
Male	1929 (63)	1063 (56)	852 (74)
Age (Years)	75 ± 8.4	73 ± 9.9	75 ± 7.3
BMI (kg/cm2)	27.6 ± 6.3	27.7 ± 7.3	27.4 ± 4.4
CAD	1153 (38)		
Type 2 diabetes	664 (22)	377 (20)	283 (25)
Hypertension	2088 (69)	1268 (68)	799 (70)
Smokers[a] (%)	1117 (37)	651 (35)	462 (41)
Lipid-lowering therapy	1314 (43)	792 (42)	512 (45)

Data are presented as mean (SD) or n (%)
a: Smoked at least once during lifetime