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# Association Between Aortic Valve Sclerosis and Clonal Hematopoiesis of Indeterminate Potential

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**Background:** The mechanism and medical treatment target for degenerative aortic valve disease, including aortic stenosis, is not well studied. In this study, we investigated the effect of clonal hematopoiesis of indeterminate potential (CHIP) on the development of aortic valve sclerosis (AVS), a calcified aortic valve without significant stenosis.

**Methods:** Participants with AVS (valves ≥2 mm thick, high echogenicity, and a peak transaortic velocity of <2.5 m/sec) and an age- and sex-matched control group were enrolled. Twenty-four CHIP genes with common variants in cardiovascular disease were used to generate a next-generation sequencing panel. The primary endpoint was the CHIP detection rate between the AVS and control groups. Inverse-probability treatment weighting (IPTW) analysis was performed to adjust for differences in baseline characteristics.

**Results:** From April 2020 to April 2022, 187 participants (125 with AVS and 62 controls) were enrolled; the mean age was 72.6±8.5 yrs, and 54.5% were male. An average of 1.3 CHIP variants was observed. CHIP detection, defined by a variant allele frequency (VAF) of ≥0.5%, was similar between the groups. However, the AVS group had larger CHIP clones: 49 (39.2%) participants had a VAF of ≥1% (vs. 13 [21.0%] in the control group; $P=0.020$), and 25 (20.0%) had a VAF of ≥2% (vs. 4 [6.5%]; $P=0.028$). AVS is independently associated with a VAF of ≥1% (adjusted odds ratio: 2.44, 95% confidence interval: 1.11–5.36; $P=0.027$). This trend was concordant and clearer in the IPTW cohort.

**Conclusions:** Participants with AVS more commonly had larger CHIP clones than age- and sex-matched controls. Further studies are warranted to identify causality between AVS and CHIP.

**Key Words:** Aortic valve sclerosis, Clonal hematopoiesis, High-throughput nucleotide sequencing, Inflammation, Variant allele frequency

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## INTRODUCTION

Clonal hematopoiesis of indeterminate potential (CHIP) occurs when clones proliferate because of specific variants in hematopoietic stem cells and blood progenitor cells in individuals without other hematologic diseases [1]. The incidence of CHIP was shown to increase with age, and patients with CHIP who had coronary artery disease and cardiovascular events more frequently had a poor prognosis [2, 3]. Additionally, *TET2*-mediated clonal hematopoiesis promoted heart failure in multiple models [4, 5]. Patients with severe aortic stenosis with *DNMT3A* and *TET2* variants, the most common drivers of CHIP, have a poor prognosis despite successful procedures [6]. However, few studies have identified an association between CHIP and valvular heart disease, particularly degenerative aortic valve disease.

Aortic valve sclerosis (AVS), defined as non-union calcification or aortic valve leaflet thickening without definite stenosis, is frequently observed in older adults, including 29% of patients aged >65 yrs old and 42% of patients aged >82 yrs old [7, 8]. Several meta-analyses have shown that patients with AVS have higher risks for cerebrovascular accidents, cardiovascular death, and all-cause death [9, 10]. Recent findings demonstrated that inflamed valves have more calcification than non-inflamed valves, suggesting that the inflammatory response is involved in aortic valve calcification [11]. However, the precise mechanism is unknown, and associations among AVS, early-stage valve degeneration, and CHIP have not been reported. We evaluated the relationship between CHIP and AVS by identifying the prevalence of CHIP in AVS patients. We also identified a potential therapeutic method for preventing aortic valve calcification.

## MATERIALS AND METHODS

### Study participants

This was a prospective age- and sex-matched, case-control study conducted at a single referral hospital (Yongin Severance Hospital, Yongin, Korea; https://trialsearch.who.int; unique identifier: KCT0005774). Patients enrolled in the case group included those who underwent transthoracic echocardiography and were diagnosed with AVS based on an aortic valve thickness of >2 mm, increased echogenicity, and a peak transaortic velocity of <2.5 m/sec (which is one condition associated with mild aortic stenosis). Participants in the control group included patients matched for sex with an age difference of <3 yrs (vs. the case group) and an aortic valve thickness of <2 mm. The blood samples for CHIP analysis were collected within three months after the participants voluntarily signed a written informed consent form. We excluded participants with: (1) hematologic disease, including hematologic malignancy; (2) insufficient extracted DNA for next-generation sequencing (NGS) analysis; or (3) more than mild aortic stenosis. We initially planned to have the same number of patients in both the case and control groups. However, participant enrollment did not go as planned because of the coronavirus disease 2019 (COVID-19) pandemic. Between January 2021 and April 2022, 125 patients and 62 age- and sex-matched controls were enrolled. This study conformed to the principles of the Declaration of Helsinki, which was revised in 2013. The Institutional Review Board of our hospital approved this study (approval number 9-2020-0082). The unique identifier of this study for clinical trial registration is KCT0005774 (URL: https://trialsearch.who.int/).

### Data collection

We prospectively collected the participants' demographic information, medical history, medication history, social history, and laboratory findings within three months of transthoracic echocardiography. Commercially available echocardiographic machines (Vivid E9/E95; GE Healthcare, Milwaukee, WI, USA) were used to obtain transthoracic echocardiographs. Conventional echocardiographic parameters and left ventricular global longitudinal strain data were collected based on established guidelines [12, 13]. The severity of AVS was classified semi-quantitatively as mild (thickness >2 mm and/or increased reflectivity on one leaflet), moderate (thickness >4 mm and increased reflectivity on one leaflet or thickness >2 mm and increased reflectivity over two leaflets), or severe (thickness >6 mm on one leaflet, thickness >4 mm, and increased reflectivity over two leaflets, or thickness >4 mm with restrictive motion over one leaflet). When classifying the severity of AVS, we excluded the zona coapta (the space in the overlapping part of the valve leaflet), which is not focal and has a diffuse thickness of approximately 2–3 mm in cases of increased echo density. We also measured the total thickness of each sclerotic lesion. Two experienced imaging cardiologists (MK and IHJ) independently confirmed the presence and severity of AVS and were unaware of information regarding the CHIP analysis when they assessed the aortic valve status. All participants were evaluated for the presence of abdominal aortic atherosclerosis, defined as a thickened and irregular intima-media wall with high echogenicity. The evaluations were performed using a cardiac sector probe (M5Sc-D, GE Healthcare; also used for transthoracic echocardiography) at the bifurcation site of both common iliac arteries or near the proximal portion.


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The aortic valve calcium score was measured using a threshold of 100 Hounsfield units and Aquaris iNtuition Edition software (version 4.4.13, TeraRecon, San Mateo, CA, USA) when participants underwent coronary computed tomography angiography.

### NGS analysis

Genomic DNA (gDNA) was extracted from EDTA-anticoagulated whole blood using a QIAsymphony DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. We selected 24 genes (*ASXL1*, *BCOR*, *CALR*, *CEBPA*, *DNMT3A*, *ETV6*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KMT2D*, *KRAS*, *MPL*, *NPM1*, *NRAS*, *RUNX1*, *SETD2*, *SF3B1*, *STAG2*, *TET2*, *TP53*, *U2AF1*, and *WT1*) associated with CHIP variants and cardiovascular diseases according to a literature review [2, 6, 14-16] and produced a custom set of target capture probes. Using 300 ng of gDNA, library generation was performed using the Twist Library Preparation EF Kit (Twist Bioscience, San Francisco, CA, USA). Target enrichment was performed using custom-designed capture probes following the manufacturer's instructions (Dxome, Seongnam, Korea). The libraries were sequenced using a NovaSeq 6000 System (Illumina, San Diego, CA, USA) to generate 2×150-bp paired-end reads, employing a unique dual index. Sequencing data were aligned to the human reference genome (hg19) using Burrows–Wheeler Aligner (BWA-mem software, version 0.7.12). Variants were identified using the PiSeq algorithm (Dxome), which was developed to verify the accuracy of molecular barcoding by computing the genome positions of mapped reads [17]. The detection limit of our method was previously validated as 0.24% for single-nucleotide variants [17]. We interpreted and classified the detected variants according to the four-tier system suggested by the standards and guidelines of the Association of Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists [18]. Variants of tiers 1, 2, or 3 were manually verified using the Integrative Genome Viewer (Broad Institute, Cambridge, MA, USA). The variant allele frequency (VAF) was calculated as a percentage by dividing the number of variant allele reads at each position by the total number of reads at that position. Variants with a VAF of <30% were defined as CHIP [19]. The study was performed in a double-anonymized manner where the researcher who conducted the NGS analysis was blinded to the participants' clinical information, including the valve status.

### Study endpoint

The primary endpoint was a difference in the proportion of CHIP variants between the AVS and control groups. Three levels of CHIP-VAF cutoffs (VAF ≥0.5%, ≥1%, and ≥2%) were used to compare the number of participants with CHIP variants between groups. We used 0.5% as the first VAF cutoff because it exceeded the assay's detection limit, which enabled us to identify variants more reliably than using the assay detection limit (VAF of 0.24%). The relative importance of the CHIP variant in the presence of AVS was compared with that of other traditional cardiovascular disease risk factors.

### Statistical analysis

The participants' baseline characteristics (continuous variables) are presented as the mean±the SD (based on Student's *t*-test) or the median (interquartile range [IQR]) (based on the Mann–Whitney *U*-test), depending on whether normality was satisfied by the Shapiro–Wilk test. We represented categorical variables, including the proportion of patients with CHIP variants and AVS, as percentages and compared them using the chi-squared test. The distribution of CHIP variants and the number of variants are presented as bar plots. The missForest algorithm was used to perform missing data imputation [20]. Logistic regression analysis was used to predict AVS based on the VAFs. Univariate and multivariate logistic regression analyses were performed to evaluate the effects of CHIP variants on the presence of AVS and traditional cardiovascular risk factors. To adjust for differences in baseline characteristics between the patient and control groups, we performed inverse-probability of treatment weighting (IPTW) analysis using a calculated propensity score (nearest neighbor method with a caliper width of 0.01). The trimming technique with upper and lower limits of 10 and 0.1, respectively, was used to stabilize the IPTW results [21]. Statistical analyses were performed using R software (version 4.1.2, R Development Core Team, Vienna, Austria), and $P<0.05$ (two-sided) was considered to reflect a statistically significant difference.

## RESULTS

### Baseline characteristics of the study participants

Among 187 participants (mean age, 72.6±8.5), 45.5% were female. No significant differences were found in the age or sex between the AVS and control groups (Table 1). Participants with AVS had higher body mass indices, hemoglobin levels, high-density lipoprotein levels, and glomerular filtration rates and tended to have hypertension and a higher level of C-reactive protein. Among the echocardiographic parameters examined, participants with AVS had higher transaortic maximal velocity and septal E/e′ (comparative rate of peak velocity of early trans-mitral

**Table 1.** Baseline clinical characteristics according to the presence of AVS

| Characteristics* | AVS (N = 125) | Age- and sex-matched control (N = 62) | $P^{\dagger}$ |
|---|---|---|---|
| Demographics | | | |
| Age, yrs | 72.9 ± 8.4 | 71.9 ± 8.5 | 0.437 |
| Female sex, N (%) | 58 (46.4) | 27 (43.5) | 0.832 |
| Body mass index, kg/m$^2$ | 25.2 ± 3.2 | 23.9 ± 3.1 | 0.013 |
| Systolic blood pressure, mmHg | 133.0 ± 18.6 | 138.1 ± 17.4 | 0.073 |
| Diastolic blood pressure, mmHg | 73.2 ± 14.3 | 73.8 ± 13.7 | 0.768 |
| Cardiovascular risk factors | | | |
| Hypertension | 102 (81.6) | 37 (59.7) | 0.002 |
| Diabetes mellitus | 52 (41.6) | 17 (27.4) | 0.083 |
| Dyslipidemia | 76 (60.8) | 28 (45.2) | 0.061 |
| Coronary artery disease | 3 (7.5) | 5 (6.7) | 0.999 |
| Chronic kidney disease | 18 (14.4) | 3 (4.8) | 0.088 |
| Previous stroke | 18 (14.4) | 3 (4.8) | 0.088 |
| Medication history, N (%) | | | |
| Antiplatelet | 58 (46.4) | 16 (25.8) | 0.011 |
| RAS blocker | 72 (57.6) | 32 (51.6) | 0.536 |
| β-blocker | 47 (37.6) | 15 (24.2) | 0.095 |
| Calcium channel blocker | 61 (48.8) | 17 (27.4) | 0.008 |
| Statins | 77 (61.6) | 32 (51.6) | 0.252 |
| Hypoglycemics | 45 (36.0) | 13 (21.0) | 0.054 |
| Anticoagulant | 19 (15.2) | 12 (19.4) | 0.610 |
| Major laboratory findings | | | |
| Hemoglobin, g/L | 124 (108–138) | 139 (128–146) | < 0.001 |
| Platelet count, × 10$^9$/L | 0.218 (0.173–0.254) | 0.231 (0.169–0.263) | 0.287 |
| GFR, mL/min/1.73 m$^2$ | 78.0 (59.0–89.5) | 85.0 (77.0–94.0) | 0.002 |
| Fasting glucose, mmol/L | 6.08 (5.38–7.41) | 5.99 (5.44–6.77) | 0.341 |
| Triglycerides, mmol/L | 1.29 (0.94–1.98) | 1.29 (0.85–1.74) | 0.518 |
| HDL, mmol/L | 1.17 (1.00–1.42) | 1.40 (1.19–1.53) | 0.007 |
| LDL, mmol/L | 2.15 (1.58–2.90) | 2.27 (1.71–3.33) | 0.208 |
| C-reactive protein, mg/L | 16 (7–77) | 8 (5–15) | 0.036 |
| NT-proBNP, pmol/L | 28.91 (8.97–181.01) | 12.69 (5.31–40.83) | 0.070 |

*Categorical variables are presented as numbers (percentages). Continuous variables are presented as the mean ± SD or median (interquartile range), as appropriate.
†$P < 0.05$.
Abbreviations: AVS, aortic valve sclerosis; RAS, renin–angiotensin system; GFR, estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration equation; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

inflow against the early diastolic velocity at the mitral annulus) values and lower septal e′ values (Supplemental Data Table S1). Participants with AVS had a higher incidence of abdominal aortic atherosclerosis (evaluated with ultrasound testing) than the age- and sex-matched control group (76.0% vs. 53.2%, $P = 0.003$).

## Association between AVS and CHIP

A high mean coverage depth (average of 58,898×) was obtained with NGS analysis of the whole blood samples of all participants. Variants in *DNMT3A* (43.2% of the AVS group and 38.7% of the control group) and *TET2* (33.6% of the AVS group and 29.0% of the control group) were most commonly detected


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in both groups (Fig. 1).

The mean number of variants between both groups was 1.6±1.5, and the AVS group exhibited more variants than the control group (1.8±1.6 vs. 1.1±1.1, $P=0.001$; Fig. 2A and 2B). The detection rates of CHIP variants with VAFs of ≥1% and ≥2% were significantly higher in the AVS group than in the control group (39.2% vs. 21.0%, $P=0.020$, and 20.0% vs. 6.5%, $P=0.028$; Fig. 2C and Table 2).

VAF≥1% and VAF≥2% were significant risk factors for predicting AVS in the patient group compared to the age- and sex-matched control group (Table 3). Multivariable logistic regression analysis revealed that the detection of CHIP variants with VAFs≥1% was independently associated with AVS, even after adjusting for various cardiovascular comorbidities, laboratory findings, and echocardiographic parameters (adjusted odds ratio [OR]: 2.44, 95% confidence interval [CI]: 1.11–5.36, $P=0.025$; Table 3). However, no association was found between the AVS severity and the detection of CHIP variants.

## IPTW analysis for adjusting baseline characteristics

As the number of patients in the case and control groups differed and the sample size was small, we performed an IPTW analysis to adjust for different baseline characteristics. Nine major clinical and laboratory variables that differed significantly between the groups were used to calculate propensity scores and perform the IPTW analysis. The baseline characteristics of the IPTW cohort were well-balanced compared with those of the original cohort (Supplemental Data Table S2 and Supplemental Data Fig. S1). The AVS group had a higher proportion of CHIP variants with a VAF of ≥1% (38.8% vs. 20.5%, $P=0.030$) or ≥2% (20.4% vs. 4.8%, $P=0.001$; Table 2). CHIP-variant carriers were significantly more likely to have AVS than non-carriers according to multivariable logistic regression analysis with adjustment for covariates, traditionally important cardiovascular risk factors, laboratory findings, and echocardiographic parameters in the IPTW cohort (OR [95% CI]: 2.45 [1.47–4.08] based on VAF≥1%, and 5.13 [2.28–11.58] based on VAF≥2%; all $P<0.01$; Table 3 and Supplemental Data Table S3).

## Validation of AVS using aortic valve calcium scores

Among the total study population, 20 patients underwent coronary computed tomography angiography; their calcium-scanning data was also available. The AVS group had significantly higher calcium scores than the control group (median [IQR]: 200.0

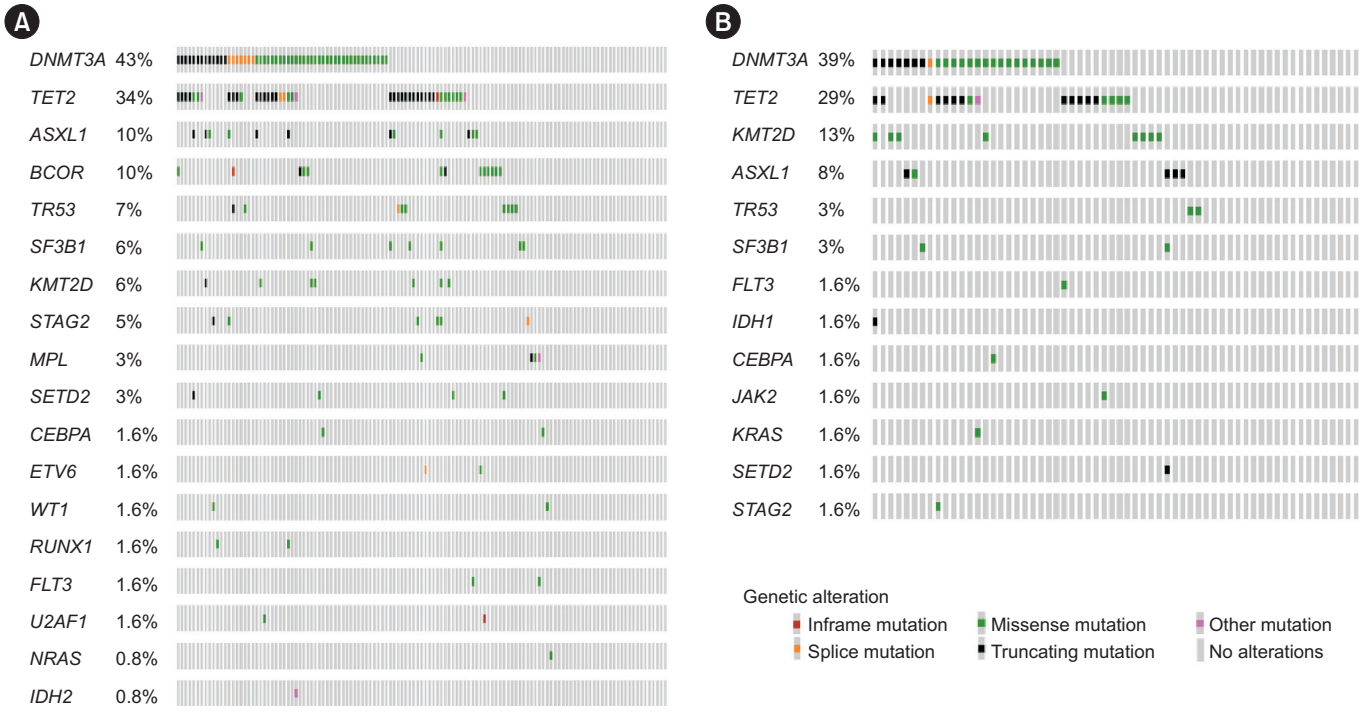


**Fig. 1.** Distribution of CHIP-associated gene variants in the study cohort. Variant frequencies in (A) the aortic valve sclerosis group and (B) the age- and sex-matched control group.
Abbreviation: CHIP, clonal hematopoiesis of indeterminate potential.

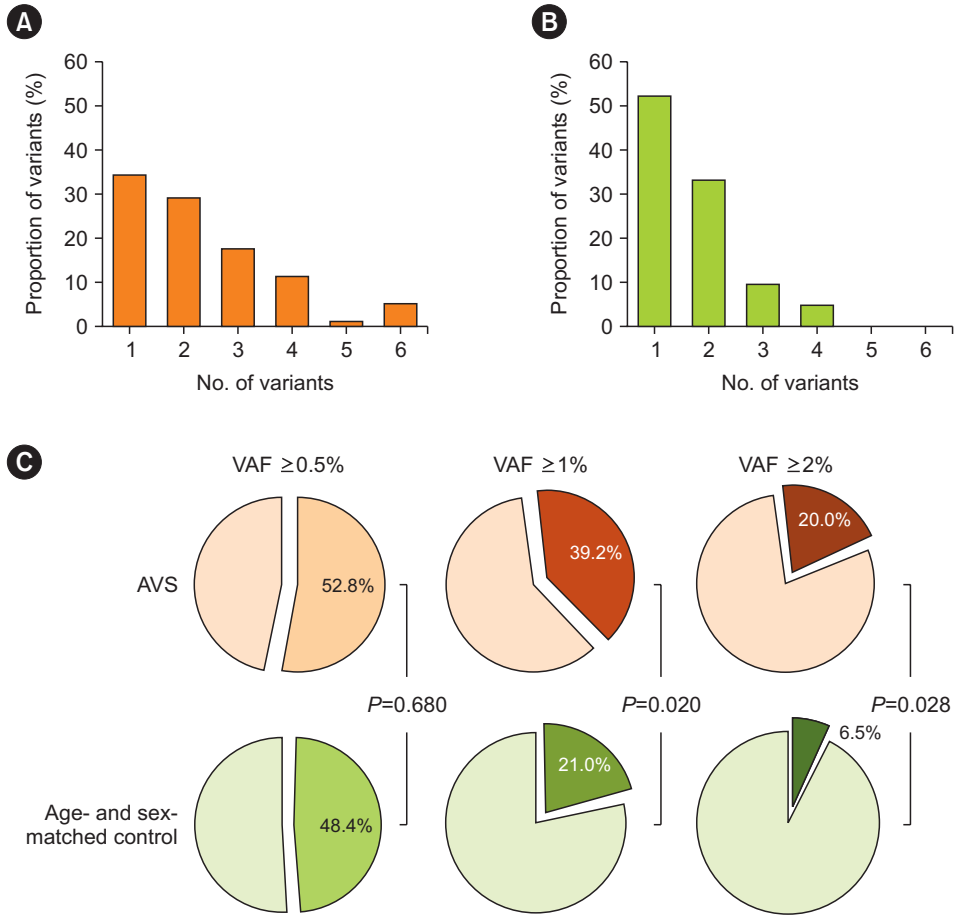


**Fig. 2.** Distributions of CHIP variants. Numbers of CHIP variants in (A) participants with aortic valve sclerosis (AVS) and (B) participants in the control group. (C) The proportions of CHIP variants in participants with AVS and in the age- and sex-matched control group, stratified by variant allele frequencies (VAFs) of ≥0.5%, ≥1%, or ≥2%.
Abbreviation: CHIP, clonal hematopoiesis of indeterminate potential.

**Table 2.** Proportions of participants with CHIP (defined as VAF ≥0.5%, ≥1%, or ≥2%) in the patient and control group (original and IPTW cohorts)

| Study cohort | Group | VAF≥0.5% | | VAF≥1% | | VAF≥2% | |
|---|---|---|---|---|---|---|---|
| | | N (%) | *P** | N (%) | *P* | N (%) | *P* |
| Original cohort | Control (N=62) | 30 (48.4) | 0.680 | 13 (21.0) | 0.020 | 4 (6.5) | 0.028 |
| | AVS (N=125) | 66 (52.8) | | 49 (39.2) | | 25 (20.0) | |
| IPTW cohort | Control (N=83) | 42 (50.6) | 0.898 | 17 (20.5) | 0.030 | 4 (4.8) | 0.001 |
| | AVS (N=98) | 52 (53.1) | | 38 (38.8) | | 20 (20.4) | |

*$P$<0.05.
Abbreviations: CHIP, clonal hematopoiesis indeterminate potential; VAF, variant allele frequency; IPTW, inverse-probability treatment weighting; AVS, aortic valve sclerosis.

[131.0–203.0] vs. 0.0 [0.0–2.4], $P$<0.001).

## DISCUSSION

The results of this prospective case-control study demonstrated a higher proportion of CHIP variants in participants with AVS than in age- and sex-matched controls (Fig. 3). The major CHIP variants detected were in *DNMT3A* and *TET2*, which commonly vary in cardiovascular diseases. CHIP variants with a VAF of ≥1% independently associated with AVS after covariate adjustment with multivariable logistic regression. IPTW analysis was performed to correct for differences in baseline characteristics, which rendered the association between AVS and the CHIP score clearer than in the original cohort. To the best of our knowledge, the current findings are the first to suggest an association between CHIP variants and AVS and that CHIP variants may af-


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**Table 3.** Logistic regression analysis for predicting AVS according to the VAF

| Model | VAF≥0.5% | | VAF≥1% | | VAF≥2% | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | *P*§ | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Model 1* | 1.19 (0.65–2.19) | 0.570 | 2.43 (1.20–4.94) | 0.014 | 3.62 (1.20–10.93) | 0.022 |
| Model 2† | 1.03 (0.52–2.04) | 0.927 | 2.44 (1.11–5.36) | 0.027 | 3.86 (1.19–12.56) | 0.025 |
| IPTW cohort‡ | 1.00 (0.64–1.57) | 0.987 | 2.45 (1.47–4.08) | <0.001 | 5.13 (2.28–11.58) | <0.001 |

*Model 1 was adjusted for age and sex.
†Model 2 was adjusted for model 1 plus hypertension, diabetes mellitus, dyslipidemia, previous stroke, glomerular filtration rate, high-density lipoprotein, C-reactive protein, NT-proBNP, septal e′, septal E/e′, and the left-atrial volume index.
‡The IPTW model was adjusted for hypertension, diabetes mellitus, dyslipidemia, previous stroke, body mass index, hemoglobin, glomerular filtration rate, high-density lipoprotein, and C-reactive protein.
§$P < 0.05$.
Abbreviations: AVS, aortic valve sclerosis; VAF, variant allele frequency; OR, odds ratio; IPTW, inverse-probability treatment weighting; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; E/e′, comparative rate of peak velocity of early trans-mitral inflow against the early diastolic velocity at the mitral annulus.

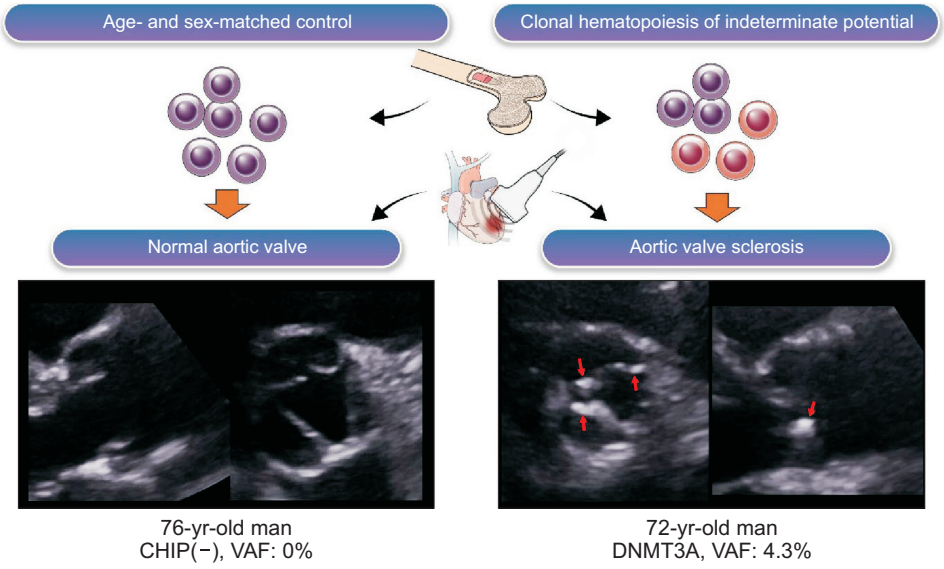


**Fig. 3.** Participants with aortic valve sclerosis (AVS) had a higher chance of having larger clonal hematopoiesis indeterminate potential (CHIP) clones than participants in the age- and sex-matched control group. Two representative cases are shown. (Left panel) A 76-yr-old man with a normal aortic valve is shown in parasternal view upon transthoracic echocardiography. He had a history of dyslipidemia and percutaneous coronary revascularization (in the left anterior descending artery) but no evidence of a CHIP variant (variant allele frequency [VAF]: 0%). (Right panel) A 72-yr-old man with AVS (red arrows) and a CHIP variant in *TET2* (VAF: 3.4%). The patient had a history of hypertension and diabetes mellitus.

fect calcified and degenerative aortic valve disease progression.

Age is a non-modifiable risk factor for cardiovascular disease [22]. The accumulation of somatic variants and the proliferation of these clones strongly correlate with age; up to 74% of CHIP variants have been observed in patients over 80 yrs of age [3, 23]. Previous data showed that increased CHIP levels significantly increased the risk of coronary artery disease and ischemic stroke, even after adjusting for clinical risk factors [3]. A large-scale patient-controlled experiment showed that high proportions of CHIP variants, particularly those in *DMNT3A*, *TET2*, *ASXL1*, and *JAK2*, were linked to coronary artery calcification, coronary artery disease, and myocardial infarction [2]. Those results showed similar trends with data from other large-scale cohorts that included other ethnicities [24, 25]. From the perspective of atherosclerosis, compared to the control group, the AVS group had more CHIP variants and more frequent abdominal aortic atherosclerosis. Previous experimental data showed that specific variants constituting CHIP help regulate circulating leu-

kocytes and cytokines [2, 5]. Previous animal data demonstrated that overactivating interleukin (IL)-1β signaling induced TET2-deficient hematopoietic cell expansion and subsequent alteration of adhesion molecules in vascular endothelial cells [26]. Additionally, *TET2* variants increased IL-1β production in the myocardium under ischemic conditions [4]. In accordance with these data, recent findings revealed a lower incidence of major adverse cardiovascular events in patients with *TET2* variant who were given IL-1β neutralizing antibodies than in those given placebo [27].

*DNMT3A*, one of the most common CHIP-associated genes linked to cardiovascular disease, causes myocardial fibrosis and decreases cardiac function by increasing macrophage accumulation and upregulating immune cell markers, such as CD68, CD3e, CD4, and CD8 [5]. In patients with chronic heart failure, *DNMT3A* variants exacerbated heart failure by enhancing the expression of a highly inflammatory transcriptome in circulating monocytes and T cells, leading to the activation of inflammatory interleukins (IL-1β, IL-6, and IL-8), the NLRP3 inflammasome, resistin, and macrophage inflammatory proteins (CCL3 and CCL4) [28]. An inflammatory gene-expression profile related to *DNMT3A* and *TET2* variants (increased expression of IL-1β, IL-6 receptor, the NLRP3 inflammasome complex, and CD163) has been consistently observed in patients with valvular heart diseases, heart failure, and coronary artery disease [29]. Higher mortality rates have also been observed in those carrying variants in CHIP-associated genes (such as *CBL*, *CEBPA*, *EZH2*, *GNB1*, *PHF6*, *SMC1A*, and *SRSF2*) among patients with chronic heart failure of ischemic origin, even when the VAFs were below 2% [30].

In terms of aortic valve disease, *TET2* or *DNMT3A* variants with a VAF of ≥2% were observed in 33.8% of patients with severe aortic stenosis [6]. We found that 20% of participants with AVS had a high percentage of CHIP variants when the VAF was ≥2%. Although the pathogenesis of aortic stenosis is unclear, several findings have suggested that valve calcification (the initial lesion) results from an inflammatory response in the valve [11, 31]. Lipoprotein (a) transports autotoxins to the aortic valve, and the autotoxin lysophosphatidic acid causes inflammation and mineralization of the valve [31]. The results of another study using $^{18}$F-NaF-based positron-emission tomography demonstrated that inflamed valves had greater calcification than non-inflamed valves, suggesting that the inflammatory response was involved in aortic valve calcification [11]. These findings support the hypothesis that an increase in CHIP variants causes valve inflammation by inflammatory cytokine overexpression, leading to valve degeneration. The results of this pilot study demonstrated an association between AVS and CHIP variants. However, further investigation is required to identify the underlying causal relationship.

AVS, defined as a condition involving structural changes such as valve thickening or increased echogenicity due to calcification of the aortic valve (but no hemodynamically proven stenosis), has been considered a benign disease or a degenerative change associated with aging in recent decades. However, recently, AVS has been recognized as a progressive disease process that leads to significant stenosis, and the results of several studies have shown that AVS can predict a poor cardiovascular or cerebrovascular prognosis [8, 32-34]. Patients with hypertension, left ventricular hypertrophy, and renal insufficiency are more likely to have AVS [35, 36]. After adjusting for cardiovascular risk factors, chronic kidney disease was not significantly associated with AVS, although mitral annular calcification was significantly associated. Mechanisms other than mineral metabolism are thought to induce AVS [37]. Similarly, in a recent well-designed randomized controlled trial, neither bisphosphonates nor denosumab (which influence bone turnover and calcium metabolism) prevented the progression of aortic stenosis [38]. A meta-analysis showed that statins were also ineffective at inhibiting the progression of AVS [39]. Contrary to our expectations, high-dose statins, which have anti-inflammatory or pleiotropic effects, may be ineffective in suppressing aortic valve inflammation. This ambiguity was also observed in a recent randomized study involving high-dose treatment with statins [40]. Considering our current results, the negative results mentioned above may indicate that statins do not directly inhibit inflammation, which is an important pathway for valve degeneration. Further studies using drugs associated with inflammatory responses caused by high CHIP variant VAFs or those that directly inhibit CHIP variants are needed to explore their effects on the progression of aortic stenosis.

Our study had some limitations. First, although this was a prospective study with age- and sex-matched patient and control groups, further studies are needed to prove a causal relationship between AVS and CHIP variants. However, our study is significant because it was the first to examine the relationship between the presence of CHIP variants and aortic valve disease. Second, this was a single-center ethnicity study. However, the CHIP component of our study was similar to that reported in previous studies on CHIP variants and cardiovascular diseases. Third, the number of patients in the case and control groups did not match equally because of the difficulty in registering patients during the COVID-19 pandemic. Fourth, although the pres-


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ence of AVS could be distinguished using echocardiography, we did not quantify valve calcification. However, some of our participants underwent computed tomography scanning simultaneously, and their valve calcium scores correlated with the AVS severity. Fifth, CHIP was analyzed using a targeted NGS panel rather than whole-genome sequencing, although our targeted NGS panel did include genes associated with recurrent cardiovascular disease. The strengths of our pilot study can serve as a basis for future aortic valve research. The results of our ultra-deep sequencing and error-correction strategy support this approach as a method for robustly detecting variants with VAFs as low as 0.5%.

In conclusion, participants with AVS had a higher chance of having larger CHIP clones than age- and sex-matched controls. CHIP with a VAF of ≥1% independently associated with AVS after adjusting for cardiovascular covariates. Further studies are warranted to identify the causality between AVS and CHIP. Interventions to suppress the CHIP variant or CHIP-mediated inflammation warrant further investigation.

## SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2023.0268

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## AUTHOR CONTRIBUTIONS

Kim M, Kim JJ, Shin S, and Jung IH designed the study. Shim Y and Lee HA performed the experiments. Kim JJ, Lee ST, Shim Y, and Lee HA performed the bioinformatics data analysis. Kim M, Kim JJ, and Shin S wrote the manuscript. Kim M, Bae S, Son NH, and Jung IH obtained the clinical samples and interpreted the clinical data. Kim M, Kim JJ, Lee ST, Shim Y, Lee HA, Bae S, Son NH, Shin S, and Jung IH reviewed and interpreted the data. Each author takes responsibility for the full content of this manuscript and approved its submission. All the authors have read and approved the final version of the manuscript.

## CONFLICTS OF INTEREST

None declared.

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