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ORIGINAL ARTICLE

FADS1 (Fatty Acid Desaturase 1) Genotype Associates With Aortic Valve FADS mRNA Expression, Fatty Acid Content and Calcification

Oscar Plunde, MD; Susanna C. Larsson, PhD; Gonzalo Artiach, MSc; George Thanassoulis, MD; Miguel Carracedo, PhD; Anders Franco-Cereceda, MD, PhD; Per Eriksson, PhD; Magnus Bäck, MD, PhD

BACKGROUND: Aortic stenosis (AS) contributes to cardiovascular mortality and morbidity but disease mechanisms remain largely unknown. Recent evidence associates a single nucleotide polymorphism rs174547 within the *FADS1* gene, encoding FADS1 (fatty acid desaturase 1), with risk of several cardiovascular outcomes, including AS. *FADS1* encodes a rate-limiting enzyme for ω-3 and ω-6 fatty acid metabolism. The aim of this study was to decipher the local transcriptomic and lipidomic consequences of rs174547 in tricuspid aortic valves from patients with AS.

METHODS: Expression quantitative trait loci study was performed using data from Illumina Human610-Quad BeadChip, Infinium Global Screening Arrays, and Affymetrix Human Transcriptome 2.0 arrays in calcified and noncalcified aortic valve tissue from 58 patients with AS (mean age, 74.2; SD, 5.9). Fatty acid content was assessed in aortic valves from 25 patients with AS using gas chromatography. Δ5 and Δ6 desaturase activity was assessed by the product-to-precursor ratio.

RESULTS: The minor C-allele of rs174547, corresponding to the protective genotype for AS, was associated with higher FADS2 mRNA levels in calcified valve tissue, whereas FADS1 mRNA and other transcripts in proximity of the single nucleotide polymorphism were unaltered. In contrast, the FADS1 Δ5-desaturase activity and the FADS2 Δ6-desaturase activity were decreased. Finally, docosahexaenoic acid was decreased in calcified tissue compared with non-calcified tissue and C-allele carriers exhibited increased docosahexaenoic acid levels. Overall desaturase activity measured with ω-3 fatty acids was higher in C-allele carriers.

CONCLUSIONS: The association between the FADS1 genotype and AS may implicate effects on valvular fatty acids.

Key Words: aortic valve ■ fatty acid desaturases ■ lipidomics ■ polymorphism, single nucleotide ■ quantitative trait loci

Calcific aortic stenosis (AS) is a common disease among elderly, affecting up to 10% of the population over the age of 80[1]. When severe, patients develop symptoms and eventually heart failure requiring surgical or transcatheter aortic valve replacement. Locally in the valve, disease progression is characterized by fibrosis and calcification,[2] involving biomechanical factors,[3] imbalance of calcification inhibitors,[4] and inflammatory processes.[5] AS also share some classical risk factors with coronary heart disease, in particular obesity,[6,7] hyperlipidemia,[8] and type 2 diabetes mellitus.[9] However, no pharmaceutical intervention has proven effective in halting AS progression.

During the last decade, genome-wide association studies have shed light on genetic risk factors revealing new loci associated with AS in addition to providing new hypotheses into the mechanisms involved.[10–12] Recent evidence states that the minor allele (C) of the single nucleotide polymorphism (SNP) rs174547 within the gene encoding fatty acid desaturase 1 (*FADS1*) is

Correspondence to: Magnus Bäck, MD, PhD, Karolinska University Hospital, Division of Valvular and Coronary Heart Disease, Huddinge M85, 141 86 Stockholm, Sweden. Email magnus.back@ki.se

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Nonstandard Abbreviations and Acronyms

AA	arachidonic acid
ALA	α-linolenic acid
AS	aortic stenosis
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
ELOVL	elongation of very long chain fatty acid protein
EPA	eicosapentaenoic acid
eQTL	expression quantitative loci
ETA	eicosatetraenoic acid
FA	fatty acid
FADS1	fatty acid desaturase 1
GLA	γ–linolenic acid
PUFA	polyunsaturated fatty acid
SNP	single nucleotide polymorphism

associated with lower risk for AS.[13] *FADS1* encodes the Δ5 desaturase enzyme, one of the rate-limiting enzymes in the endogenous synthesis of polyunsaturated fatty acids (PUFAs),[14] depicted in Figure I in the Data Supplement. Within the same locus are also found FADS2, responsible for the Δ6 desaturase activity, and FADS3, whose activity is less known. Δ5 desaturase catalyzes the insertion of a double bond at carbon 5 yielding the ω6-fatty acid (FA), arachidonic acid (AA; 20:4ω6) from dihomo-γ-linolenic acid (DGLA; 20:3ω6), and the ω3-FA eicosapentaenoic acid (20:5ω3) from eicosatetraenoic acid (20:4ω3). Δ6 desaturase catalyzes double bond insertion at carbon 6 yielding the ω6-FA γ–linolenic acid (GLA; 18:3ω6) from linolenic acid (18:2ω6) and the ω3-FA stearidonic acid (18:4ω3) from α-linolenic acid (ALA; 18:3ω3).[14]

In general, the ω3-PUFA eicosapentaenoic acid is a precursor for an anti-inflammatory response, and the ω6-pathway AA is a precursor for an inflammatory response.[15] Previous genome wide association studies have found an association between *FADS1* genotypes and fatty acid composition in plasma.[16] In addition, variants within the *FADS1* locus have been associated with Δ5 and Δ6 desaturase activity measured by the ratio of AA to DGLA and GLA to linolenic acid, respectively.[17,18] Furthermore, the activity of the desaturases has been associated with inflammation,[18] type 2 diabetes mellitus,[19] and coronary artery disease.[20] Previous studies have shown that SNPs may affect neighboring genes,[21] making it important to study the gene expression effect on genes in proximity of a SNP. To date, the impact of the *FADS1* genotype rs174547 on mRNA expression, lipid profile, and calcification locally in stenotic tricuspid aortic valves has not been studied. Therefore, the aim of the present study was to assess this question by an expression quantitative loci (eQTL) study and profiling of PUFAs locally in human aortic valves in relation to FADS genotype.

MATERIALS AND METHODS

IRB approval was obtained, according to the guidelines noted in Instructions to Authors on the AHA website. All methods used in the study is available in the Data Supplement. Informed consent was obtained from all human subjects. The investigation was approved by the Ethical Committee of Northern Stockholm and was in agreement with the Declaration of Helsinki. The material used in this study (including data, analytical tools, etc) will be made available to other researchers for the purposes of reproducing the results or replicating the procedures (available at the authors' laboratories).

RESULTS

FADS1 Genotype Alters FADS2 but Not FADS1 mRNA Levels in Aortic Valves

Given that a SNP may affect genes other than the expected, we performed an eQTL to determine the effect of rs174547 on genes ±200 kb, in human aortic

Table 1. Characteristics for 58 Patients Included in the eQTL Analysis

	rs174547			
	CC n=8	CT n=17	TT n=33	*P* Value
Age	75.3 (2.5)	74.1 (6.7)	73.2 (6.0)	0.674
Male sex, no.	7	9	28	0.031
Never smoked, no.	3	8	16	0.169
Body mass index (SD)	27.2 (3.5)	27.4 (3.7)	29.5 (5.1)	0.205
Ejection fraction >55, no.	7	9	19	0.139
CAD, no.	5	8	23	0.295
CVD, no.	7	16	30	0.85
ASA 75 mg, no.	6	10	18	0.574
Ang blockers, no.	3	10	19	0.555
β Blockers, no.	5	6	20	0.202
Ca-antagonist, no.	3	4	9	0.765
Diuretics, no.	1	5	18	0.047
Statins, no.	4	6	25	0.17
eGFR <60, no.	5	6	13	0.82
MAP (SD)	102.7 (8.6)	96.7 (10.9)	95.9 (8.2)	0.175
HbA1c (SD)	36.2 (4.0)	39.0 (11.5)	42.4 (9.8)	0.273
Vmax	4.5 (0.53)	4.3 (0.53)	4.4 (9.51)	0.617

Continuous data are presented as mean (SD). Categorical data are presented as number of patients carrying the trait. *P*-values stems from ANOVA test on continuous data and χ^2 test on categorical data. Coronary artery disease (CAD), cardiovascular disease (CVD), acetylic salicylic acid (ASA), Ang blockers (angiotensin inhibitors) including angiotensin receptor inhibitors and angiotensin-converting enzyme inhibitors. eGFR (estimated glomerular filtration rate) was assessed with Cystatin C measurement the day before surgery and expressed as mL/min per 1.73 m^2. Mean arterial pressure (MAP) derived from blood pressure measurement the day before surgery. For HbA1c, data were available for n=6 in CC, n=16 in CT and n=32 in TT group. Vmax (peak aortic jet velocity), measured preoperatively with trans thoracic echocardiography, were available for n=8 in CC, n=16 in CT, and n=32 in TT.

valves. Fifty-eight patients with severe tricuspid valve AS were included in the eQTL, of which 8 patients were homozygotes for the minor allele (CC), 17 were heterozygotes (CT), and 33 were homozygotes for the common allele (TT). Only number of males and number of patients on diuretics differed significantly between the genotype groups (Table 1). Of 18 genes included in the eQTL, solely the expression of *FADS2* in calcified tissue significantly correlated with the *FADS1* genotype after FDR adjustment (Table I in the Data Supplement). FADS1 mRNA levels did not differ significantly based on genotype, but a nonsignificant trend toward increased expression in T-allele carriers was observed in resilient and thickened tissue (Figure 1A). The levels of FADS2 mRNA in calcified tissue decreased significantly in presence of the T-allele (R=–0.40; q=0.03) and a trend to a similar pattern was observed in noncalcified tissue (Figure 1B).

FADS mRNA Expression Is Decreased in Calcified Tissue

Recent evidence dictates that Δ5-desaturase, based on the SNP rs174547 within *FADS1*, is associated with AS.[13] Calcification of the aortic valve is a hallmark in AS and to test the hypothesis that expression of the FADS genes associate with calcification, the mRNA levels of FADS1 and FADS2 were assessed and compared in noncalcified (resilient and thickened) and calcified human tricuspid aortic valve tissue. Lower expression in calcified compared with noncalcified tissue (Figure 2) was observed with a fold change for FADS1 and FADS2 of 0.60 and 0.75, respectively (P<0.0001). A similar pattern was observed with each genotype (Figure 1).

FADS1 Genotype Correlates With Δ5-Desaturase FADS1 Activity in Calcified Aortic Valves

To assess the effect of the *FADS1* genotype on Δ5-desaturase activity locally in the valve, gas chromatography analysis of PUFA composition was carried out. FADS1 Δ5-desaturase activity was determined by the ratio of 2 ω6-FAs, AA to DGLA. In the study sample with calcified aortic valve tissue (n=25 patients with AS), 4 patients were homozygotes (CC) for the allele of rs174547, which is inversely associated with AS. This genotype was associated with a significantly lower valvular FADS1 Δ5-desaturase activity compared with the tissue derived from carriers of the common allele (TT; n=15). Calcified valve tissue derived from carriers of heterozygous alleles (n=6 patients) presented an intermediate phenotype. Considering all 3 groups, FADS1 genotype correlated in an allele-dependent manner with a rho coefficient of 0.575 (P=0.003; Figure 3A). Noncalcified aortic valve tissue was obtained from 16 AS patients and exhibited a similar pattern, albeit not reaching statistical significance (Figure 3B).

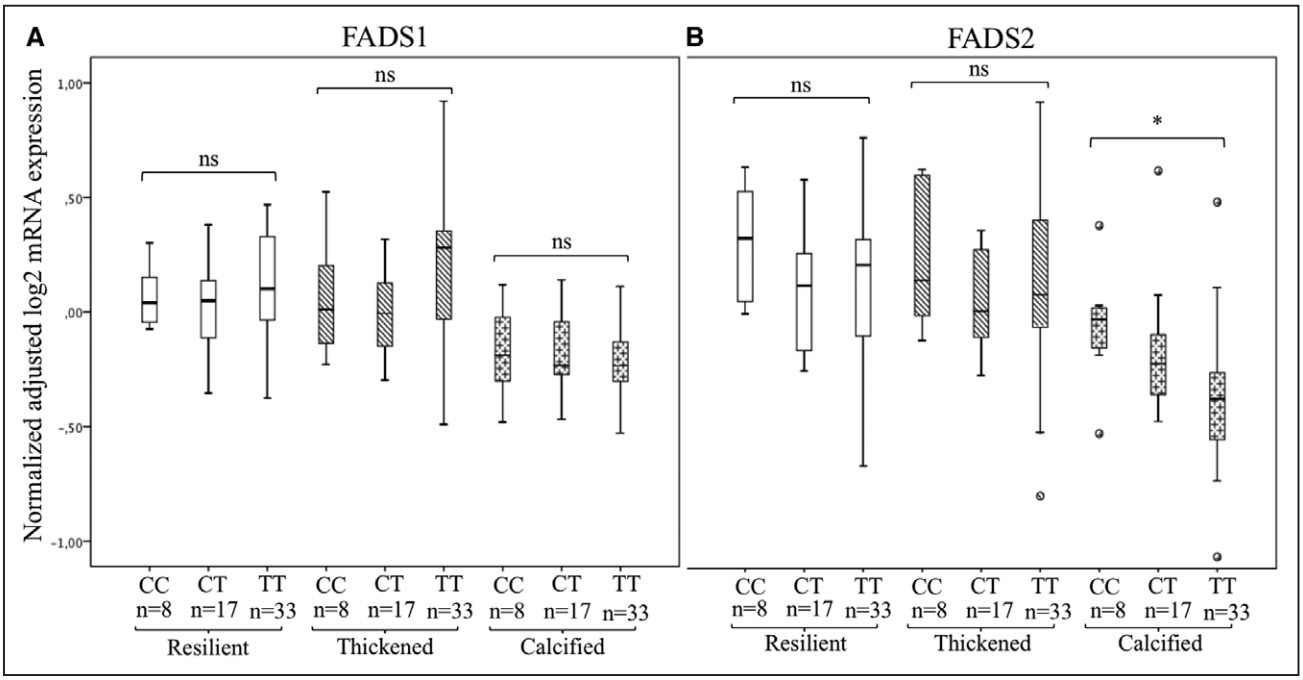

Figure 1. Association of FADS mRNA expression with snp rs174547 in calcified and noncalcified human aortic valve tissue. Normalized mRNA expression of (**A**) FADS1 (fatty acid desaturase 1) and (**B**) FADS2. CC, CT, and TT represent genotype of the snp rs 174547 in resilient, thickened, and calcified tissue. Correlations and *P*-values results from a linear regression model adjusting for age and sex. Boxplots show the median, interquartile range, and outliers (outside the 95% CI). Error bars represent 95% CI. * indicates *P* value <0.05.

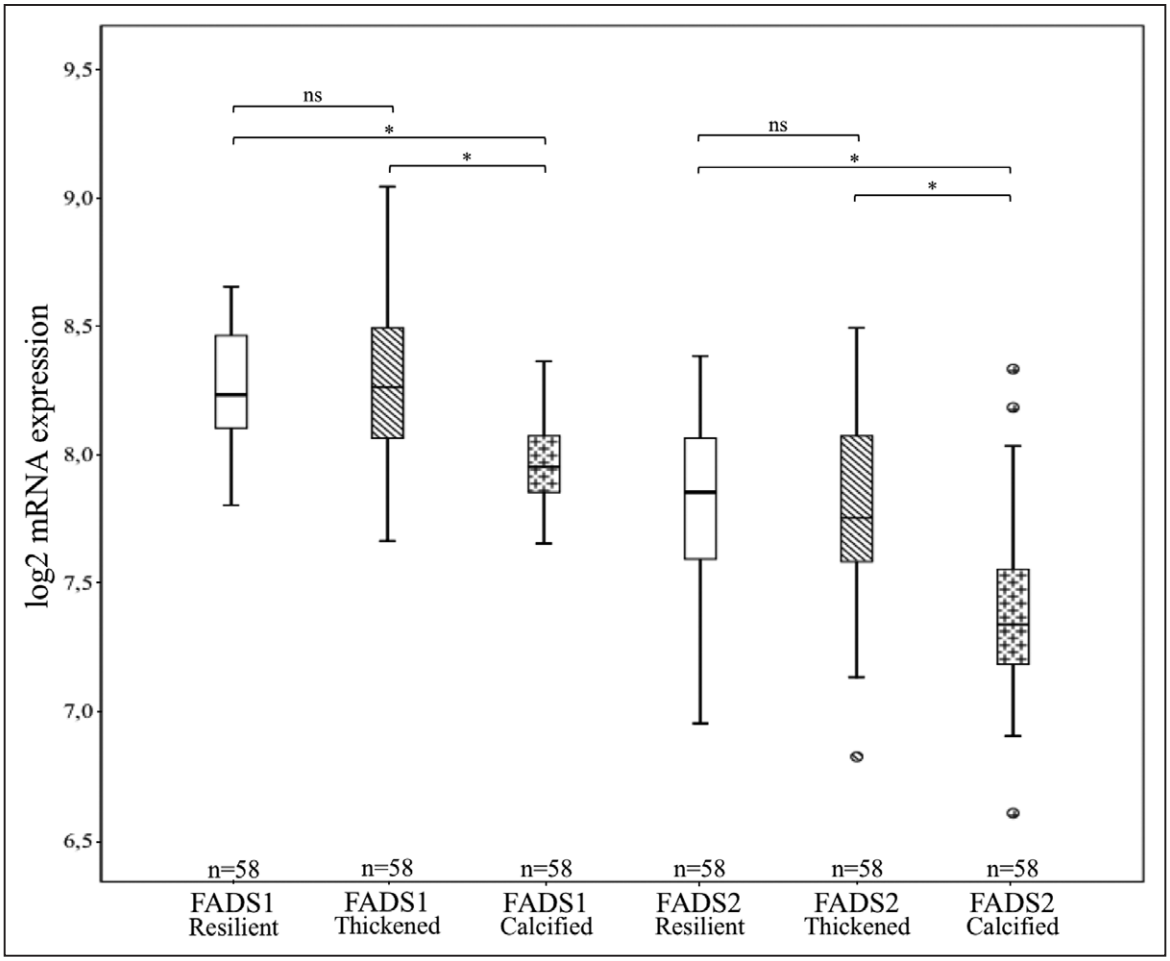

Figure 2. FADS1 (fatty acid desaturase 1)/2 expression in calcified and noncalcified human aortic valve tissue.
FADS1 and FADS2 log2mRNA expression in resilient, thickened, and calcified tissue. Repeated measures ANOVA followed by Bonferroni post hoc tests was used to assess difference in expression between the tissue types. Boxplots show the median, interquartile range and outliers (outside 95% CI). Error bars represent 95% CI. § indicates *P* value <0.0001, ns indicates no significance.

FADS2 Δ6-Activity in Calcified Aortic Valves Is Associated With FADS1 Genotype

Our eQTL results indicate an effect on FADS2 expression based on FADS1 genotype rs174547. To assess whether the FADS1 genotype also affects FADS2 Δ6-desaturase activity locally in the valve, Δ6-desaturase activity was determined by the ratio of 2 ω6-FAs, GLA to linolenic acid. A pattern similar to the Δ5-desaturase activity was observed with a significant decrease in FADS2 Δ6-desaturase activity in the samples from CC-carriers of rs174547 and a significant allele-dependent correlation with a rho of 0.593 (*P*=0.002; Figure 3C). The same pattern was observed in noncalcified tissue (n=16) with a rho of 0.590 (*P*=0.016; Figure 3D).

FADS1 Genotype Is Associated With Fatty Acid Content in Human Aortic Valves

In support of a broader effect of the *FADS1* genotype rs174547 on the local PUFA profile in aortic valves, we show that several PUFAs within both the ω3 and ω6 classes were altered according to genotype (Table 2). In calcified tissue, significant inverse correlations with genotype were established for eicosadienoic acid (EDA), DGLA, and docosahexaenoic acid (DHA; Figure 4) with the lowest levels in valve tissue from T-allele carriers. In contrast, GLA was positively correlated with the T-allele in both calcified and noncalcified tissue (Figure 4). Only the associations with DGLA and DHA resisted Bonferroni correction for multiple univariate analyses.

Differential Fatty Acid Composition in Calcified and Non-Calcified Aortic Valve Tissue

Given the observation that FADS1/2 expression is changed in calcified tissue, we sought to investigate if composition of PUFAs was altered in calcified compared with noncalcified tissue. Human aortic tricuspid valves from 13 patients with AS used for the fatty acid analysis contained both noncalcified and calcified tissue (paired samples) and were used to determine how valvular PUFA composition

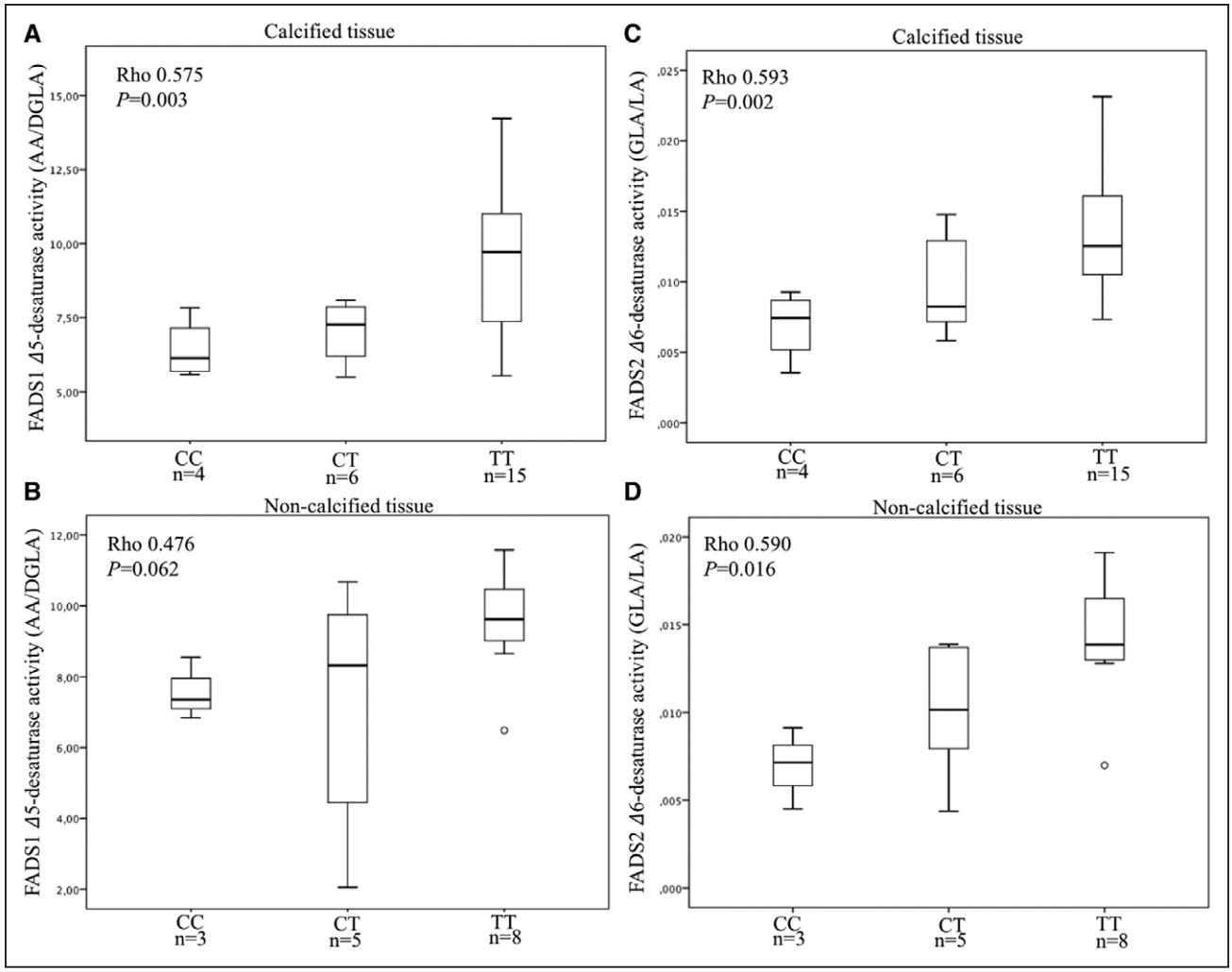

Figure 3. Association of FADS1 (fatty acid desaturase 1) genotype with Δ5 and Δ6-activity in human aortic valves..
Ratio of arachidonic acid (AA) to dihomo-γ-linolenic acid (DGLA) determine the ω6 FADS1 Δ5-desaturase activity and ratio of γ-linolenic acid (GLA) to linolenic acid (LA) determine the ω6 FADS2 Δ6-desaturase activity. This was performed in calcified (**A** and **C**) and noncalcified (**B** and **D**) human tricuspid aortic valve tissue. Correlation coefficients and *P* values are results from Spearman-Rho. Boxplots show the median, interquartile range, and outliers (outside 95% CI). Error bars represents 95% CI.

change with calcification. When comparing the PUFAs showing correlation with FADS genotype and the activity of FADS1/2, only FADS2 Δ6-desaturase activity, GLA and DHA differed between calcified and noncalcified tissue. FADS2 activity and GLA were higher in calcified tissue, fold change 1.15 (95% CI, 1.04–1.26) and 1.24 (95% CI, 1.02–1.45), respectively, whereas ω3-PUFA DHA was lower in calcified compared with noncalcified tissue with a fold change of 0.81 (95% CI, 0.67–0.95; Figure 5).

FADS1 Genotype Is Associated With Overall FADS Δ4, Δ5, and Δ6-Desaturase Activity of the ω-3 Pathway Leading to DHA Synthesis in Human Aortic Valves

In addition to FADS2 Δ6-desaturase activity, FADS2 also catalyzes the last step of DHA biosynthesis from ALA.[22] Given the observation of increased FADS2 transcripts (Figure 1B) and increased DHA levels (Figure 4D) in human aortic valves derived from carriers of the CC genotype associated with lower risk of AS,[13] we finally assessed the overall FADS Δ4, Δ5, and Δ6-desaturase activity by means of the DHA to ALA ratio in human aortic valves. The results indicated a recessive pattern with similar ratios in the CT and TT genotypes, whereas valve tissue derived from carriers of the CC genotypes exhibited a significantly higher DHA/ALA in both calcified (Figure 6A; *P*=0.009) and noncalcified tissue (Figure 6B; *P*=0.03).

DISCUSSION

The present study identified an increase in FADS2 mRNA levels in calcified human aortic valve tissue

Table 2. Correlations of Subjected Fatty Acids With the T-Allele of SNP rs174547 in Aortic Valve Tissue

	Calcified Tissue (n=25)		Noncalcified Tissue (n=16)	
	Rho	*P* Value	Rho	*P* Value
LA (18:2ω6)	−0.185	0.376	0.014	0.958
EDA (20:2ω6)	−0.442	0.027	−0.444	0.085
GLA (18:3ω6)	0.439	0.028	0.729	0.00136
DGLA (20:3ω6)	−0.563	0.003	−0.299	0.261
AA (20:4ω6)	−0.028	0.894	0.085	0.754
DTA (22:4ω6)	−0.066	0.755	−0.114	0.674
ALA (18:3ω3)	−0.199	0.340	−0.008	0.976
EPA (20:5ω3)	−0.153	0.465	−0.034	0.901
DPA (22:5ω3)	−0.359	0.078	−0.169	0.532
DHA (22:6ω3)	−0.606	0.00134	−0.389	0.136

Ten fatty acids were included in the correlation analysis between genotype rs174547 and fatty acid composition in human tricuspid aortic valves. Correlation coefficients and *P* values are results from Spearman-Rho where a negative Rho indicate decrease in T-allele carriers (vs C). AA indicates arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, dodecylthioacetic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; and LA, linolenic acid.

from carriers of the minor C allele of *FADS1* rs174547, shown to confer decreased risk of AS.[13] We also confirmed that the previously described systemic genotype-dependent decreased FADS1 activity, also was present locally in human calcified aortic valves and that this was accompanied by significant genotype-dependent change in valvular lipid profiles. In contrast, on the ω3-pathway, the lower AS risk genotype exhibited higher overall FADS desaturase activity and higher DHA content. Taken together, these findings indicate that the *FADS1* genotype conferring decreased AS risk is associated with increased FADS2 transcription in calcified aortic valves, and an increased valvular desaturase activity on the ω 3-pathway in human aortic valves leading to increased DHA content.

The minor C allele of the *FADS1* rs174547 was associated with a lower risk of AS with an odds ratio of 0.92 (95% CI, 0.83–0.99). rs174547 is located in an intron within the FADS1 gene in position 61570783 on chromosome 11 (Figure II in the Data Supplement). The FADS1 gene is located between position 61567097 to 61647626 and FADS2 gene is located on the between position 61583728 to 61634826. As the location of the SNP is within the FADS1 gene, focus has primarily been on this transcript. However, the eQTL analysis in the present study identified only FADS2 mRNA levels as being significantly associated with the FADS1 genotype. This is in line with publicly available data from the Genotype-Tissue Expression (GTEx) where several types of tissue showing increased FADS2 mRNA expression with C-allele (Table II in the Data Supplement). In contrast, none of the other 18 transcripts located within 200 kb of the SNP rs174547, including FADS1 mRNA levels, exhibit differential expression depending on genotype in any of the types of aortic valve tissue studied. This finding contrasts with human liver, where the C allele of rs174547 is associated with lower FADS1 gene expression[23] in addition to other tissues included in the GTEx (Table II in the Data Supplement) and illustrates the importance of studying the local changes in tissues associated with observed outcomes in genetic association studies. The downregulation of FADS1 mRNA in calcified tissue within each genotype suggests that other mechanisms of repressed FADS1 mRNA may obscure genotype-dependent transcriptional regulation in calcified tissue.

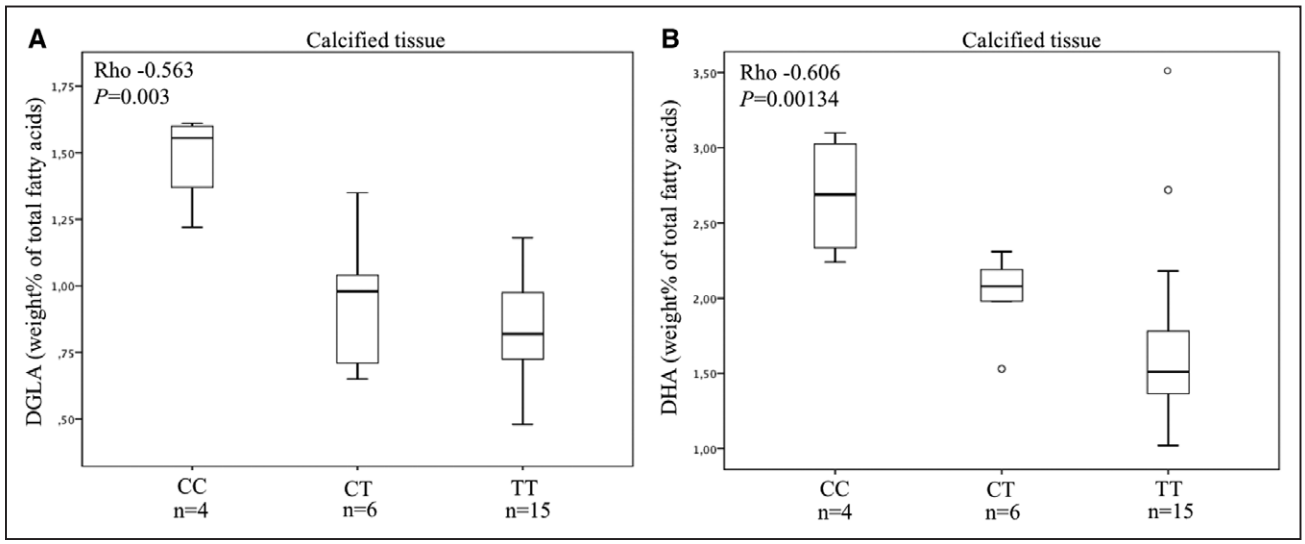

Figure 4. Association of FADS1 (fatty acid desaturase 1) genotype with fatty acids in human aortic valves.
Significant negative correlations between (**A**) dihomo-γ-linolenic acid (DGLA; Rho 0.563; *P*=0.003) and (**B**) docosahexaenoic acid (DHA; Rho 0.606; *P*=0.001) and FADS1 genotype rs174547. Correlation coefficients and *P* values are results from Spearman-Rho. Boxplots show the median, interquartile range, and outliers (outside 95% CI). Error bars represents 95% CI.

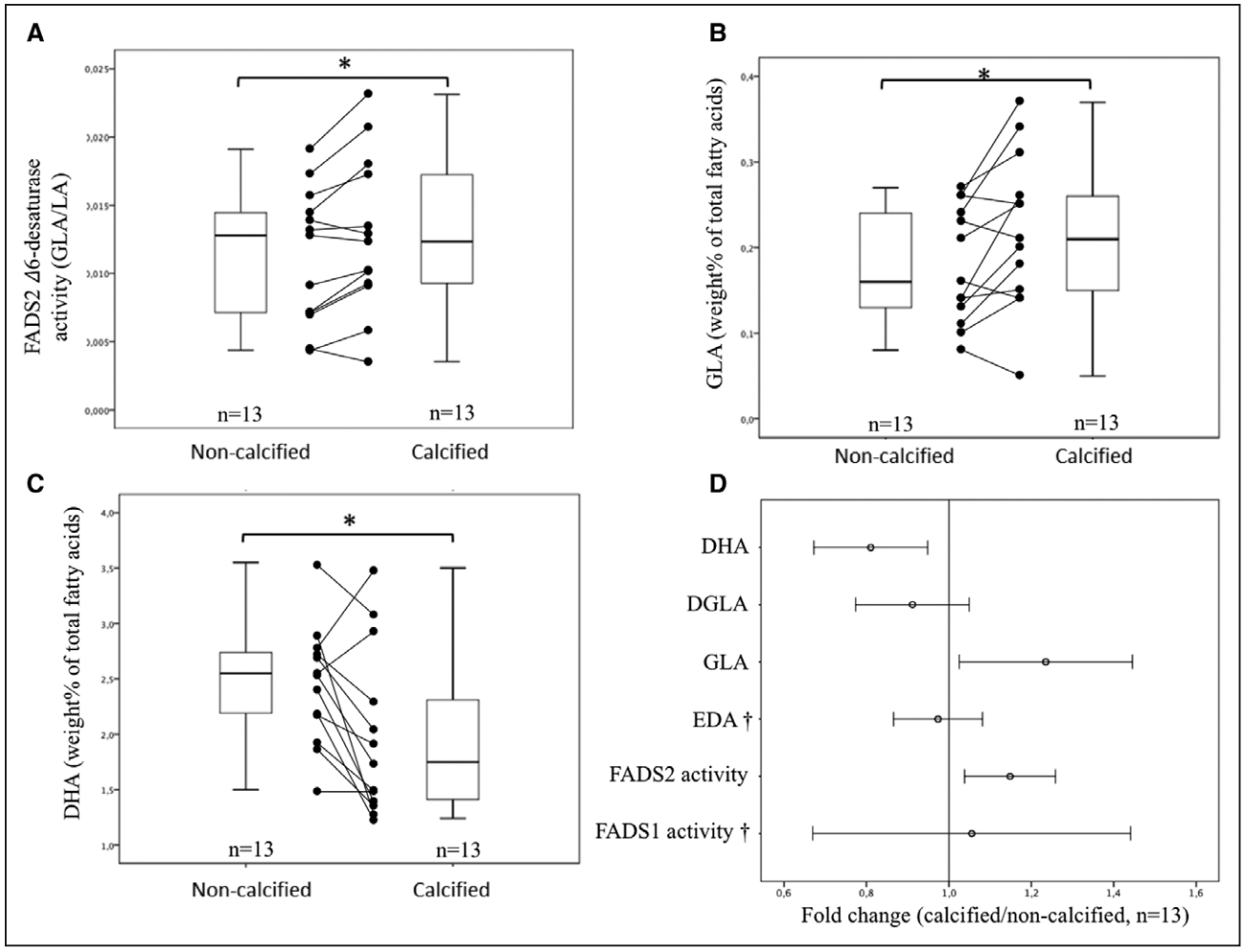

Figure 5. Change in fatty acids (FAs) in calcified and noncalcified tissue.
Significant differences between calcified and noncalcified human tricuspid aortic valve tissue. **A**, FADS2 Δ*6* activity measured by product to precursor ratio, that is, γ-linolenic acid/linolenic acid (GLA/LA). **B**, γ-linolenic acid and (**C**) docosahexaenoic acid (DHA). Paired *t*-test was used for parametric data (FADS2 activity, log10DGLA, log10GLA, log10DHA) and Wilcoxon signed-rank test was used for non-normally distributed data (eicosadienoic acid [EDA], FADS1 [fatty acid desaturase 1] activity). Mean fold change for all tested FAs and activities with 95% CI, DHA 0.81 (0.67–0.95), dihomo-γ-linolenic acid (DGLA) 0.91 (0.77–1.05), GLA 1.24 (1.02–1.45), EDA 0.97 (0.87–1.08), FADS2 Δ6 activity 1.15 (1.04–1.26), and FADS1 Δ5 activity 1.10 (0.67–1.4). Paired samples are shown, and boxplots show the median, interquartile range, and outliers (outside 95% CI). Error bars represents 95% CI. * indicates *P* value <0.05. † indicates the use of nonparametric paired test.

A SNP may carry out biological effects in a variety of different ways depending on location. A majority of the SNPs are located in noncoding regions, which is the case for FADS1 genotype rs174547 which is an intronal SNP. Introns generally contain cis-acting (acting nearby) regulatory elements, which explains why this SNP can affect more than merely FADS1.[24] Although it is beyond the scope of this study to investigate, one can speculate that the SNP might affect mRNA splicing or an regulatory element which affect FADS2 expression.[25]

The importance of both the FADS1 and FADS2 pathway in valve calcification was further supported by their downregulation in calcified aortic valve tissue as compared with resilient and thickened tissue. It is therefore of particular interest that genotype-dependent FADS2 expression was significant only in calcified aortic valve tissue in the present study. Although these findings are consistent with other eQTL analyses that a SNP may affect genes outside of its genetic locus,[21] rs174547 in *FADS1* is correlated with functional SNPs (rs174576 and rs174583) in *FADS2*,[26] which may have contributed to the observed association.

Previous studies have shown that *FADS1* genotype alters systemic FADS1 activity, with a reduced Δ5-desaturase activity associated with the minor C allele.[27,28] The present study extends those findings by showing a decreased FADS1 Δ5-desaturase activity locally in the calcified aortic valve tissue derived from carriers of the minor C allele, assessed on by ratio of the ω 6-PUFAs AA/DGLA. This observation was driven by an increase in DGLA, whereas AA was not significantly different. This contrast previous findings in plasma from normal and overweight patients showing decreased AA in C-allele carriers.[29] Our observation suggests that genotype-dependent

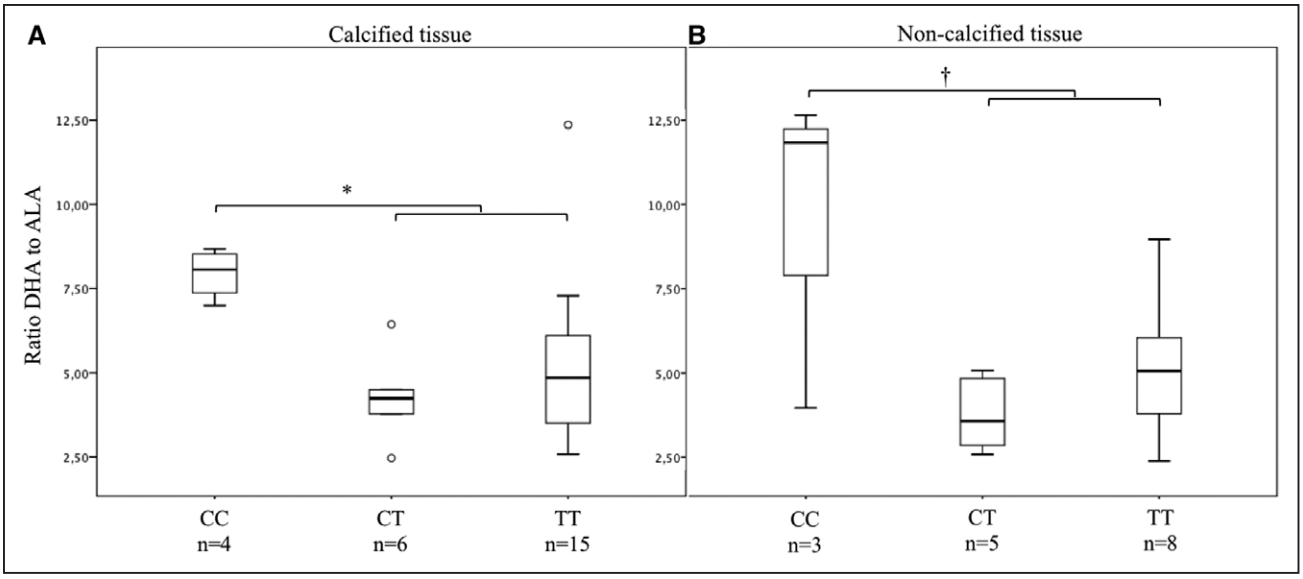

Figure 6. Overall FADS Δ4, Δ5, and Δ6-desaturase activity of the ω3 pathway.
To determine Δ4, Δ5, and Δ6-desaturase activity of the ω3 pathway, ratio of docosahexaenoic acid (DHA) to ALA was analyzed in 25 calcified and 16 noncalcified human tricuspid aortic valve tissue. CC, CT, and TT refers to genotype of rs174547. The difference between group CC and CT/TT was assessed with unpaired *t*-tests. Boxplots show the median, interquartile range, and outliers (outside 95% CI). Error bars represents 95% CI. **P*-value <0.01 and †*P*-value <0.05.

biological effects locally in the aortic valve either does not involve AA to a significant extent, or that AA is further metabolized by valvular cyclo- and lipoxygenases to pro-inflammatory lipid mediators. It should however also be taken into consideration that DGLA is not only dependent on Δ5-desaturase activity but also on activity of enzymes upstream such as ELOVL (elongation of very long chain fatty acid protein) and Δ6 and Δ8 desaturase activity carried out by FADS2-encoded enzymes.[14]

The present study also detected decreased FADS2 Δ6-desaturase activity with the C-allele in both calcified and noncalcified tissue. The latter observation was in contrast to the increase in FADS2 mRNA levels observed in valves derived from C-allele carriers. However, it should be noted that we did not detect any significant changes (after Bonferroni correction) in the individual ω6-fatty acids determined by FADS2 activity. In fact, the only fatty acid, which exhibited a genotype-dependent valvular abundance in addition to DGLA was the ω3-fatty acid DHA. Of these, only DHA exhibited significant changes both when comparing calcified versus noncalcified tissue and showed correlation with the FADS1 genotype in calcified tissue.

The observed genotype-dependent alterations of DHA and FADS2 mRNA in calcified tissue support increased levels in carriers of C allele in rs174547. Importantly, FADS2 also catalyzes a Δ4-desaturase activity to yield DHA.[22] Indeed, when including all steps of desaturase activity by DHA to ALA ratio, we detected a significantly increased ratio in calcified aortic valve tissue from C-allele carriers. Taken together, these results point to that the minor C allele, associated with lower AS risk, associates with higher FADS2 transcripts contributing to higher DHA in calcified human aortic valves.

AA is the substrate for proinflammatory prostaglandins, thromboxanes, and leukotrienes, all which have been associated with disease processes leading to AS.[30,31] In contrast, DHA serve as the substrate for a class of lipid mediators that are anti-inflammatory and mediate the resolution of inflammation. Collectively, these have been coined specialized pro-resolving mediators, and include the D-series of resolvins, maresins, and protectins.[15] Our data show lower FADS2 expression and DHA in calcified aortic valves from T-allele carriers and that DHA is decreased in calcified versus noncalcified tissue. Hence, specialized pro-resolving mediators may partake in the mechanism behind the observation that C-allele carriers associates with a decreased risk of AS in addition to abdominal aortic aneurysm and coronary artery.[13]

In this study, only human aortic valves deemed tricuspid by the operating surgeon were used. This is a profound strength as the pathology differ between bicuspid and tricuspid aortic valve disease.[32,33] We consider the number of patients included in the eQTL a strength. However, taking the GTEx data into consideration, we cannot rule out the possibility that significant results could have been obtained for FADS1 mRNA levels in a larger cohort. The number of patients included in the FA analysis is however low. More specifically, increased number of patients carrying the minor allele C would undoubtedly have led to more power in the statistical tests. Unfortunately, it was not possible to measure FADS1/2 activity specifically in the ω3-pathway as the precursor/product PUFAs for this activity were not assessed. However, we

provide an alternative using DHA to ALA ratio with the drawback of involving other enzymatic steps not involving FADS enzymes. Furthermore, the fact that several enzymes, such as ELOVLs affect the fatty acid metabolism (Figure I in the Data Supplement), somewhat limits its conclusions drawn on FADS activity based on ratios. Dietary intake of PUFAs is an important factor determining at least the fatty acid profile in blood[34] and potentially also locally in aortic valves. Given that no information regarding diet was available for the patients in the present study, it was not possible to control for differences in intake of certain PUFAs. Likewise, although cardiac fatty acid content is reflected by their systemic levels,[35] the correlation of circulating and heart valve fatty acids is unknown. It should also be mentioned that all valve tissues came from patients having AS, which may bias the genotype-phenotype associations. Finally, because of the observational design of this study, final conclusions about causality could not be drawn.

In summary, we present novel data on the effect of *FADS1* rs174547 in human aortic valves, unexpectedly showing that only FADS2 mRNA expression in calcified tissue was affected by the genotype by means of higher expression in the minor C-allele, corresponding to the protective genotype for AS. Despite a genotype-dependent increase in FADS1 and FADS2 activity in calcified aortic valve tissue from carriers the T-allele, we observed decreased levels of DHA. Moreover, DHA was decreased in calcified tissue compared with noncalcified tissue and overall desaturase activity in the ω-3 pathway was higher in C-allele carriers. Therefore, we conclude that the association between the FADS1 genotype and lower risk for AS may implicate DHA and DHA-derived specialized pro-resolving mediators that contribute to a protective effect.

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Affiliations

Department of Medicine Solna, Unit of Cardiovascular Medicine (O.P., G.A., M.C., P.E., M.B.), Unit of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine (S.C.L.), and Department of Molecular Medicine and Surgery (A.F.-C.), Karolinska Institutet, Stockholm. Department of Surgical Sciences, Uppsala University, Sweden (S.C.L.). Division of Experimental Medicine, McGill University Health Centre, Montreal, QC, Canada (G.T.). Theme Heart and Vessels, Division of Valvular and Coronary Disease, Karolinska University Hospital, Stockholm, Sweden (A.F.-C., M.B.).

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Disclosures

None.

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