FADS1 Genotype Associates with Aortic Valve FADS mRNA Expression, Fatty Acid Content and Calcification

Running title: Plunde et al.; FADS1 and aortic stenosis

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Abstract:

**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 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 AS patients.

**Methods** - Expression quantitative trait loci (eQTL) study was performed using data from Illumina Human610-Quad BeadChip, Infinium Global Screening Arrays, and Affymetrix Human Transcriptome 2.0 arrays in calcified and non-calcified aortic valve tissue from 58 AS patients (mean age 74.2, SD 5.9). Fatty acid content was assessed in aortic valves from 25 AS patients 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 SNP were unaltered. In contrast, the FADS1 Δ5-desaturase activity and the FADS2 Δ6-desaturase activity were decreased. Finally, docosahexaenoic acid (DHA) was decreased in calcified tissue compared to non-calcified tissue and C-allele carriers exhibited increased DHA 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 stenosis; fatty acid; omega3 fatty acids, expression quantitative trait loci
Nonstandard abbreviations and Acronyms

AS - Aortic stenosis
GWAS - Genome wide association studies
SNP - Single nucleotide polymorphism
FADS1 - Fatty acid desaturase 1
PUFAs - Polyunsaturated fatty acids
FA - Fatty acid
AA - arachidonic acid [20:4ω6]
DGLA - dihomo-γ-linolenic acid [20:3ω6]
EPA - eicosapentaenoic acid [20:5ω3]
ETA - eicosatetraenoic acid [20:4ω3].
GLA - γ-linolenic acid [18:3ω6]
LA - linolenic acid [18:2ω6]
SDA - stearidonic acid [18:4ω3]
ALA - α-linolenic acid [18:3ω3]
eQTL - expression quantitative loci
ELOVL - elongation of very long chain fatty acid protein
Introduction

Calcific aortic valve stenosis (AS) is a common disease among elderly, affecting up to 10% of the population over the age of 80\textsuperscript{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\textsuperscript{2}, involving biomechanical factors\textsuperscript{3}, imbalance of calcification inhibitors\textsuperscript{4} and inflammatory processes\textsuperscript{5}. AS also share some classical risk factors with coronary heart disease, in particular obesity\textsuperscript{6,7}, hyperlipidemia\textsuperscript{8}, and type 2 diabetes mellitus\textsuperscript{9}. However, no pharmaceutical intervention has proven effective in halting AS progression.

During the last decade, genome wide association studies (GWAS) have shed light on genetic risk factors revealing new loci associated with AS in addition to providing new hypotheses into the mechanisms involved\textsuperscript{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 (\textit{FADS1}) is associated with lower risk for AS\textsuperscript{13}. \textit{FADS1} encodes the \(\Delta5\) desaturase enzyme, one of the rate-limiting enzymes in the endogenous synthesis of polyunsaturated fatty acids (PUFAs)\textsuperscript{14}, depicted in Supplementary Fig 1. Within the same locus are also found \textit{FADS2}, responsible for the \(\Delta6\) desaturase activity, and \textit{FADS3}, whose activity is less known. \(\Delta5\) desaturase catalyzes the insertion of a double bond at carbon 5 yielding the \(\omega6\)-fatty acid (FA) arachidonic acid (AA) [20:4\(\omega6\)] from dihomo-\(\gamma\)-linolenic acid (DGLA) [20:3\(\omega6\)] and the \(\omega3\)-FA eicosapentaenoic acid (EPA) [20:5\(\omega3\)] from eicosatetraenoic acid (ETA) [20:4\(\omega3\)]. \(\Delta6\) desaturase catalyzes double bond insertion at carbon 6 yielding the \(\omega6\)-FA \(\gamma\)-linolenic acid (GLA) [18:3\(\omega6\)] from linolenic acid (LA) [18:2\(\omega6\)] and the \(\omega3\)-FA stearidonic acid (SDA) [18:4\(\omega3\)] from \(\alpha\)-linolenic acid (ALA) [18:3\(\omega3\)]\textsuperscript{14}. 
In general, the ω3-PUFA EPA is a precursor for an anti-inflammatory response and the
ω6-pathway AA is a precursor for an inflammatory response\textsuperscript{15}. Previous GWAS have found an
association between \textit{FADS1} genotypes and fatty acid composition in plasma\textsuperscript{16}. In addition,
variants within the \textit{FADS1} locus have been associated with Δ5 and Δ6 desaturase activity
measured by the ratio of AA to DGLA and GLA to LA respectively\textsuperscript{17, 18}. Furthermore, the
activity of the desaturases has been associated with inflammation\textsuperscript{18}, type 2 diabetes\textsuperscript{19}, and
coronary artery disease\textsuperscript{20}. Previous studies have shown that SNPs may affect neighboring
genes\textsuperscript{21}, making it important to study the gene expression effect on genes in proximity of a SNP.
To date, the impact of the \textit{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.

\textbf{Material 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 supplemental data. 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 expression quantitative trait loci (eQTL) to determine the effect of rs174547 on genes +/- 200kb, in human aortic valves. 58 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 (Supplementary Table 1). FADS1 mRNA levels did not differ significantly based on genotype, but a non-significant trend towards increased expression in T-allele carriers was observed in resilient and thickened tissue (Fig 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 non-calcified tissue (Fig 1B).

**FADS mRNA expression is decreased in Calcified Tissue**

Recent evidence dictates that \(\Delta 5\)-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 non-calcified (resilient and thickened) and calcified human tricuspid aortic valve tissue. Lower expression in calcified compared to non-calcified tissue (Fig 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 (Fig 1).
**FADS1 genotype correlates with Δ5-desaturase FADS1 Activity in Calcified Aortic Valves**

In order 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 two ω6-FAs, AA to DGLA. In the study sample with calcified aortic valve tissue (n=25 AS patients), 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 three groups, FADS1 genotype correlated in an allele-dependent manner with a rho coefficient of 0.575 (P=0.003; Figure 3A). Non-calcified aortic valve tissue was obtained from 16 AS patients and exhibited a similar pattern, albeit not reaching statistical significance (Figure 3B).

**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 two ω6-FAs, GLA to LA. 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; Fig 3C). The same pattern was observed in non-calcified tissue (n=16) with a rho of 0.590 (P=0.016; Fig 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 DHA (Fig 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 non-calcified tissue (Fig 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 non-calcified tissue. Human aortic tricuspid valves from 13 AS patients used for the fatty acid analysis contained both non-calcified and calcified tissue (paired samples) and were used to determine how valvular PUFA composition 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 non-calcified 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 to non-calcified tissue with a fold change of 0.81 (95% CI 0.67, 0.95) (Fig 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. Given the observation of increased FADS2 transcripts (Fig 1B) and
increased DHA levels (Fig 4D) in human aortic valves derived from carriers of the CC genotype associated with lower risk of AS\textsuperscript{13} we finally assessed the overall FADS $\Delta 4$, $\Delta 5$, and $\Delta 6$-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 (Fig 6A; $P=0.009$) and non-calcified tissue (Fig 6B; $P=0.03$).

**Discussion**

The present study identified an increase in FADS2 mRNA levels in calcified human aortic valve tissue from carriers of the minor C allele of *FADS1* rs174547, shown to confer decreased risk of AS.\textsuperscript{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 $\omega 3$-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 $\omega 3$-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 (Supplementary Figure 2). The FADS1 gene is located between position 61567097 to 61647626 and FADS2 gene is located on the between position 61583728 to 61634826. Since 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 (Supplementary Table
2). In contrast, none of the other 18 transcripts located within 200kb 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\textsuperscript{23} in addition to other tissues
included in the GTEx (Supplementary Table 2) 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.

A SNP may carry out biological effects in a variety of different ways depending on
location. A majority of the SNPs are located in non-coding 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.\textsuperscript{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.\textsuperscript{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, rs174547 in FADS1 is correlated with functional SNPs (rs174576 and rs174583) in FADS2, 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. 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 ω6-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. Our observation suggests that genotype-dependent 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 proinflammatory 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 elongation of very long chain fatty acid protein (ELOVL) and Δ6 and Δ8 desaturase activity carried out by FADS2 encoded enzymes.

The present study also detected decreased FADS2 Δ6-desaturase activity with the C-allele in both calcified and non-calcified 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 vs non-calcified 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.\textsuperscript{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.\textsuperscript{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 (SPMs), and include the D-series of resolvins, maresins and protectins.\textsuperscript{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 non-calcified tissue. Hence, SPMs 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.\textsuperscript{13}

In this study, only human aortic valves deemed tricuspid by the operating surgeon were used. This is a profound strength since the pathology differ between bicuspid and tricuspid aortic valve disease.\textsuperscript{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 since 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 (Supplementary Fig 1) somewhat limits 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\textsuperscript{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\textsuperscript{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 \textit{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 to non-calcified 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 SPMs that contribute to a protective effect.

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Table 1. Characteristics for 58 patients included in the eQTL analysis

|                      | rs174547 |          |          |          |     |
|----------------------|----------|----------|----------|----------|-----|
|                      | CC n=8   | CT n=17  | TT n=33  |          | P   |
| 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 75mg – no.       | 6        | 10       | 18       | 0.574   |
| Ang blockers – no.   | 3        | 10       | 19       | 0.555   |
| Betablockers – 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 (standard deviation). Categorical data are presented as number of patients carrying the trait. P-values stems from analysis of variance (ANOVA) test on continuous data and chi square test on categorical data. CAD (Coronary artery disease), CVD (cardiovascular disease), ASA (acetylic salicylic acid), 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 prior to surgery and expressed as mL/min/1.73 m². MAP (mean arterial pressure) derived from blood pressure measurement the day prior to surgery. For HbA1c, data was 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.
Table 2. Correlations of Subjected Fatty Acids with the T-allele of SNP rs174547 in Aortic Valve Tissue

|                          | Calcified tissue (n=25) | Non-calcified tissue (n=16) |
|--------------------------|-------------------------|-----------------------------|
|                          | Rho         | P    | Rho         | P    |
| 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|

10 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). LA (linolenic acid), EDA (eicosadienoic acid), GLA (γ-linolenic acid), DGLA (dihomo-γ-linolenic acid), AA (arachidonic acid), DTA (dodecylthioacetic acid), ALA (α-linolenic acid), EPA (eicosapentaenoic acid), DPA (docosapentaenoic acid) and DHA (Docosahexaenoic acid).
Figure Legends:

**Figure 1.** Association of FADS mRNA expression with snp rs174547 in calcified and non-calcified human aortic valve tissue. Normalized mRNA expression of (A) FADS1 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% confidence interval). Error bars represent 95% confidence interval (CI). * indicates p-value <0.05.

**Figure 2.** FADS1/2 expression in calcified and non-calcified human aortic valve tissue. FADS1 and FADS2 log2mRNA expression in resilient, thickened, and calcified tissue. Repeated measures analysis of variance 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% confidence interval). Error bars represent 95% confidence interval (CI). § indicates p-value <0.0001, ns indicates no-significance.

**Figure 3.** Association of FADS1 Genotype with Δ5 and Δ6-Activity in human aortic valves. Ratio of AA to DGLA determine the ω6 FADS1 Δ5-desaturase activity and ratio of GLA to LA determine the ω6 FADS2 Δ6-desaturase activity. This was performed in calcified (A and C) and non-calcified (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% confidence interval). Error bars represents 95% CI. AA (arachidonic acid), DGLA (dihomo-\(\gamma\)-linolenic acid), GLA (\(\gamma\)-linolenic acid) and LA (linolenic acid).

**Figure 4.** Association of FADS1 genotype with fatty acids in human aortic valves. Significant negative correlations between (A) DGLA (Rho 0.563, \(p=0.003\)) and (B) 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% confidence interval). Error bars represents 95% confidence interval. DGLA (dihomo-\(\gamma\)-linolenic acid), DHA (docosahexaenoic acid).

**Figure 5.** Change in fatty acids (FAs) in calcified and non-calcified tissue. Significant differences between calcified and non-calcified human tricuspid aortic valve tissue. A) FADS2 \(\Delta 6\) activity measured by product to precursor ratio i.e. \(\gamma\)-linolenic acid to linolenic acid (GLA/LA). B) \(\gamma\)-linolenic acid (GLA) and C) Docosahexaenoic acid (DHA). Paired \(t\)-test was used for parametric data (FADS2 activity, \(\log_{10}\text{DGLA}, \log_{10}\text{GLA}, \log_{10}\text{DHA}\)) and Wilcoxon signed rank test was used for non-normally distributed data (EDA, FADS1 activity). Mean fold change for all tested FAs and activities with 95% confidence interval (CI), DHA 0.81 (0.67-0.95), dihomo-\(\gamma\)-linolenic acid (DGLA) 0.91 (0.77-1.05), GLA 1.24 (1.02-1.45), EDA (eicosadienoic acid) 0.97 (0.87-1.08), FADS2 \(\Delta 6\) activity 1.15 (1.04-1.26) and FADS1 \(\Delta 5\) 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 non-parametric paired test.
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 DHA to ALA was analyzed in 25 calcified and 16 non-calcified 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% confidence interval). Error bars represents 95% confidence interval. *P-value <0.01 and †P-value <0.05.
Figure 1: Box plots showing the changes in fatty acid desaturase (FADS) activity and GLA (γ-linolenic acid) content between non-calcified and calcified samples.

A. FADS2 Δ6 desaturase activity (GLA/LA).

B. GLA (weight % of total fatty acids).

C. DHA (weight % of total fatty acids).

D. Graph showing fold change in FADS2 and FADS1 activity between calcified and non-calcified samples (n=13).

- Fold change (calcified/non-calcified, n=13)
  - DHA
  - DGLA
  - GLA
  - EDA
  - FADS2 activity
  - FADS1 activity

Legend:
- * Indicates significant difference.
- n=13 for both non-calcified and calcified samples.
