Acetylsalicylic Acid Is Associated With a Lower Prevalence of Ascending Aortic Aneurysm and a Decreased Aortic Expression of Cyclooxygenase 2

Carl Granath, MD*; David Freiholtz*; Fredrik Bredin, MD, PhD; Christian Olsson, MD, PhD; Anders Franco-Cereceda, MD, PhD; Hanna M. Björck, PhD

BACKGROUND: Acetylsalicylic acid (ASA) therapy has been associated with a reduced prevalence and growth rate of abdominal as well as intracranial aneurysms, but the relationship between ASA and ascending aortic aneurysm formation remains largely unknown. The aim of the present study was to investigate whether ASA therapy is associated with a lower prevalence of ascending aortic aneurysm in a surgical cohort.

METHODS AND RESULTS: One thousand seven hundred patients undergoing open-heart surgery for ascending aortic aneurysm and/or aortic valve disease were studied in this retrospective cross-sectional study. Aortic dilatation was defined as an aortic root or ascending aortic diameter ≥45 mm. Medications were self-reported by the patients in a systematic questionnaire. Cyclooxygenase gene expression was measured in the intima-media portion of the ascending aorta (n=117). In a multivariable analysis, ASA was associated with a reduced prevalence of ascending aortic aneurysm (relative risk, 0.68 [95% CI, 0.48–0.95], P=0.026) in patients with tricuspid aortic valves, but not in patients with bicuspid aortic valves (relative risk, 0.93 [95% CI, 0.64–1.34], P=0.687). Intima-media cyclooxygenase expression was positively correlated with ascending aortic dimensions (P<0.001 for cyclooxygenase-1 and P=0.05 for cyclooxygenase-2). In dilated, but not nondilated tricuspid aortic valve aortic specimens, ASA was associated with significantly lower cyclooxygenase-2 levels (P=0.034).

CONCLUSIONS: Our findings are consistent with the hypothesis that ASA treatment may attenuate ascending aortic aneurysmal growth, possibly via cyclooxygenase-2 inhibition in the ascending aortic wall and subsequent anti-inflammatory actions.

Key Words: ascending aortic aneurysm ■ aspirin ■ COX-2

Acute ascending aortic aneurysm (AscAA) is a progressive potentially life-threatening disease of the ascending aorta with multiple causes. Medial degeneration in combination with a low-grade inflammation is a common feature of some, but not all types of AscAA. For example, aortopathy in patients with bicuspid aortic valves (BAVs) does not seem to be driven by inflammatory factors, but rather being a consequence of a dysfunctional endothelium1 and/or a repair deficiency.2,3 The diverse pathogenic mechanisms behind AscAA development and progression remains, however, poorly understood, and treatment strategies to prevent or slow AscAA growth are lacking.

Acetylsalicylic acid (ASA) therapy has emerged as a promising pharmaceutical agent in the management of aneurysmal disease. Specifically, an inverse relation between low-dose ASA therapy and expansion rate of medium-sized abdominal aortic aneurysms (AAA) has
been shown.\(^4\) Recently, in a large systematic review and meta-analysis,\(^5\) low-dose ASA treatment was also associated with reduced risk of growth of unruptured intracranial aneurysm. Moreover, animal models support a protective role of ASA on aneurysmal expansion and rupture,\(^6–8\) specifically via cyclooxygenase 2 and prostaglandin inhibition,\(^9–11\) and targeting this pathway has been shown to attenuate AAA development.\(^7,12–13\)

In the present study, the relation between ASA therapy and AscAA was evaluated in a surgical cohort of patients undergoing elective open-heart surgery for aortic valve disease and/or AscAA repair.

**METHODS**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Patients**

A total of 1700 patients (n=808 patients with tricuspid aortic valve [TAV], n=892 patients with BAV) aged ≥18 years and undergoing elective open-heart surgery for aortic valve disease and/or AscAA at the Karolinska University Hospital, Solna, Sweden between February 2007 and August 2021 were included in the study. Patients with monogenic forms of AscAA (10 TAV and 6 BAV), diabetes (98 TAV and 108 BAV), and missing data regarding ASA therapy (7 TAV and 3 BAV) were excluded from the analysis, resulting in a final study population of 1468 individuals (693 TAV and 775 BAV) (see flowchart in Figure 1). Patients with diabetes were excluded from the analyses because of the protective association between diabetes and aneurysm formation,\(^14\) and thus a potentially differential underlying pathogenic mechanism. The study was approved by the Regional Research Ethics Approval Committee in Stockholm (application no. 2006/78431/1 and 2012/1633-31/4) and was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from all 1700 patients initially screened.

All patients underwent intraoperative transesophageal echocardiography, and the maximum ascending aortic diameter was measured leading edge to leading edge in end-diastole at the level of the aortic root (anulus, sinus of Valsalva, sinotubular junction) and the tubular segment. Aortic dilatation was defined as an aortic root or ascending aortic diameter ≥45 mm, based on current guidelines for management of patients with thoracic aortic disease.\(^15\) Tissue biopsies were collected from the anterior part of the proximal aorta. In patients undergoing isolated aortic valve procedures, a ≤2×5 cm\(^2\) full-thickness aortic wall biopsy was recovered from the site of the aortotomy. No complications
were reported related to this sampling procedure. The intima-medial layer was separated from the adventitia before storage in the RNA stabilizing solution RNAlater (Invitrogen) at −80 °C. Body surface area was calculated using the Mosteller formula using height and weight measurements recorded preoperatively. Medications and comorbidities were self-reported via a study questionnaire, which was completed together with a research nurse. High-sensitivity C-reactive protein levels were assessed before surgery as a measure of systemic inflammation.

Valve disease was classified as either aortic stenosis or aortic insufficiency based on the primary surgical indication. Hypertension was defined as a diagnosis of hypertension and treatment with any class of antihypertensive medication. Known vascular disease included a diagnosis of any form of ischemic heart disease, peripheral arterial disease, or cerebrovascular disease, as well as angiographic evidence of significant coronary atherosclerosis. Anticoagulant medications included warfarin, apixaban, rivaroxaban, and dabigatran.

mRNA Extraction and Gene Expression Analysis

Total mRNA was extracted from the intima-medial portion using RNeasy kit (Qiagen), according to the manufacturer’s instructions. Gene expression was measured in 117 consecutive patients from which good-quality RNA was obtained (RNA integrity number ≥6). (20 TAV with dilatation (D), 23 TAV without dilatation (ND), 43 BAV-D and 31 BAV-ND), using the Affymetrix GeneChip Human Exon 1.0 ST array and protocols. All expression measurements were RNA normalized and log2 transformed before analysis. The arrays have previously been validated by quantitative real-time polymerase chain reaction for 11 genes.

Statistical Analysis

Continuous variables are presented as mean±SD. Nominal variables were summarized using frequencies and proportions. Independent samples t test with Welch’s correction was used to assess differences in continuous variables between individuals with and without ASA treatment. The χ² test, or Fisher exact test when appropriate, compared differences in nominal variables. A modified Poisson regression model with robust error variance was used to estimate relative risk of aortic dilatation associated with ASA treatment with adjustment for potential confounding variables. All statistical analyses were performed using SPSS, version 25.0 (IBM), with cases stratified according to valve type (BAV and TAV) because of the known differences in disease mechanism. A 2-tailed P value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

In total, 693 patients with TAV and 775 patients with BAV were analyzed. Clinical characteristics of all patients are shown in Table 1. In short, patients on ASA therapy were older (P<0.001 for both TAV and BAV patients), were more frequently treated with antihypertensive medication (P<0.001 for both TAV and BAV patients) and lipid-lowering agents (P<0.001 for both TAV and BAV patients), and more often diagnosed with vascular disease (P<0.001 for both TAV and BAV patients). Moreover, there were more male patients with TAV on ASA therapy than without ASA (P=0.036). Among patients with BAV, there were more smokers among ASA-treated individuals (P=0.009). Concurrent treatment with both ASA and anticoagulants was uncommon (Table 1).

The prevalence of AscAA was 30.9% (n=214) in patients with TAV and 28.0% (n=217) in patients with BAV, respectively, predominantly involving the tubular segment. In patients with TAV, but not BAV, ASA therapy was associated with smaller aortic dimensions (at the sinus of Valsalva, sinotubular junction and ascending aorta, P<0.001 for all 3 locations), and a lower prevalence of aortic root and/or ascendance aneurysm (P<0.001) (Table 1).

ASA Treatment Is Associated With a Lower Prevalence of AscAA in Patients With TAV But Not BAV

To further evaluate the impact of ASA treatment on the prevalence of aortic dilatation in patients with TAV and BAV, respectively, a modified Poisson regression model was used, including known potentially confounding covariates (age, sex, body surface area, known vascular disease, treated hypertension, chronic inflammatory disease, valve disease, anticoagulants, other thrombocyte inhibitors, and lipid-lowering medication). The analysis showed that in patients with TAV, preoperative ASA therapy was associated with a significantly lower prevalence of AscAA at the time of surgery (relative risk, 0.68 [95% CI, 0.48–0.95], P=0.026), whereas in patients with BAV, no such association could be found (relative risk, 0.93 [95% CI, 0.64–1.34], P=0.687) (Table 2).

Increased Cyclooxygenase 2 Expression in Dilated Aortic Tissue of Patients With TAV

To further investigate the observed inverse association between ASA therapy and aortic dilatation in patients with TAV, and the possible influence on cyclooxygenase signaling in the ascending aortic wall, gene expression
First, cyclooxygenase-1 and cyclooxygenase-2 gene expression levels were measured in nondilated and dilated aortic specimen. As shown in Figure 2, both cyclooxygenase-1 and cyclooxygenase-2 expression correlated positively with ascending aortic dimensions.

Table 1. Patient Characteristics

| Variable                  | Patients with TAV | Patients with BAV | P value | Patients with TAV | Patients with BAV | P value |
|---------------------------|-------------------|-------------------|---------|-------------------|-------------------|---------|
| Male sex, n (%)           | 293 (63.6)        | 166 (71.6)        | 0.036   | 452 (74.2)        | 126 (75.9)        | 0.659   |
| Age, y (SD)               | 67.7 (10.7)       | 71.6 (8.1)        | <0.001  | 57.5 (13.4)       | 63.3 (9.7)        | <0.001  |
| BSA, m² (SD)              | 1.98 (0.24)       | 1.98 (0.21)       | 0.906   | 2.0 (0.22)        | 1.99 (0.23)       | 0.655   |
| hsCRP, mg/L (SD)          | 3.4 (7.8)         | 3.6 (7.5)         | 0.697   | 2.22 (5.2)        | 2.14 (3.7)        | 0.935   |
| HDL-cholesterol, mmol/L (SD) | 1.4 (0.4)     | 1.4 (0.5)         | 0.615   | 1.4 (0.4)         | 1.4 (0.4)         | 0.721   |
| LDL-cholesterol, mmol/L (SD) | 2.7 (1.0)     | 2.6 (2.5)         | 0.466   | 2.9 (0.9)         | 2.6 (1.0)         | 0.071   |
| Treated hypertension, n (%) | 338 (73.3)   | 198 (85.3)        | <0.001  | 317 (52.1)        | 121 (72.9)        | <0.001  |
| Known vascular disease, n (%) | 99 (21.5)    | 134 (57.8)        | <0.001  | 58 (9.5)          | 71 (42.8)         | <0.001  |
| Chronic inflammatory disease, n (%) | 33 (7.3)   | 22 (9.5)          | 0.372   | 36 (6.2)          | 11 (6.7)          | 0.810   |
| Current smoking, n (%)    | 22 (7.0)         | 11 (6.8)          | 0.968   | 45 (7.5)          | 23 (14.0)         | 0.009   |
| Lipid-lowering agents, n (%) | 149 (32.5)  | 153 (65.1)        | <0.001  | 134 (22.0)        | 94 (56.6)         | <0.001  |
| Corticosteroids, n (%)    | 16 (3.3)         | 10 (4.3)          | 0.617   | 11 (1.8)          | 1 (0.6)           | 0.478*  |
| Anticoagulants, n (%)     | 93 (20.3)        | 7 (3.0)           | <0.001  | 55 (9.0)          | 2 (1.2)           | 0.001   |
| Other thrombocyte inhibitors | 43 (9.4)    | 13 (5.5)          | 0.078   | 21 (3.4)          | 5 (3)             | 0.782   |
| Aortopathy                |                  |                   |         |                   |                   |         |
| Sinus of Valsalva, mm (SD) | 37.4 (8.0)     | 34.5 (8.4)        | <0.001  | 35.7 (5.8)        | 35.9 (5.2)        | 0.273   |
| Sinotubular junction, mm (SD) | 32.1 (7.7)   | 28.9 (6.0)        | <0.001  | 31.1 (5.8)        | 30.8 (5.4)        | 0.210   |
| Ascending, mm (SD)        | 39.5 (10.5)      | 35.6 (8.0)        | <0.001  | 39.3 (7.8)        | 39.2 (8.5)        | 0.292   |
| Root and/or ascending aortic dilatation, n (%) | 178 (38.9) | 36 (15.8)        | <0.001  | 173 (28.9)        | 44 (27)           | 0.627   |
| Isolated root dilatation, n (%) | 45 (8.8)   | 6 (2.6)           | 0.068   | 13 (2.2)          | 1 (0.6)           | 0.323*  |
| Aortic dilatation without valve disease, n (%) | 53 (11.6) | 8 (3.4)           | <0.001  | 21 (3.4)          | 6 (1.2)           | 0.409   |
| Valve disease             |                  |                   |         |                   |                   |         |
| Aortic valve stenosis, n (%) | 173 (37.5)  | 159 (68.5)        | <0.001  | 411 (67.5)        | 133 (80.1)        | 0.002   |
| Aortic valve insufficiency, n (%) | 224 (48.6) | 66 (28.4)        | <0.001  | 191 (31.4)        | 31 (18.7)         | 0.001   |

ASA indicates acetylsalicylic acid; BSA, body surface area; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; and TAV, tricuspid aortic valve.

*Fisher exact test.

Table 2. ASA Therapy and Ascending Aortic Dilatation in Patients With TAV and BAV, Multivariable Analysis

| Variable                  | Patients with TAV | Patients with BAV | P value | Patients with TAV | Patients with BAV | P value |
|---------------------------|-------------------|-------------------|---------|-------------------|-------------------|---------|
| Age                       | 1.01 (1.00–1.02)  | 0.152             |         | 1.02 (1.01–1.03)  | 0.001             |         |
| BSA (0.1 m²)              | 1.63 (0.94–2.82)  | 0.084             |         | 2.54 (1.34–4.82)  | 0.004             |         |
| Male sex                  | 1.06 (0.80–1.43)  | 0.677             |         | 1.07 (0.74–1.54)  | 0.720             |         |
| Known vascular disease    | 1.13 (0.83–1.54)  | 0.434             |         | 0.76 (0.49–1.18)  | 0.210             |         |
| Treated hypertension      | 1.11 (0.82–1.50)  | 0.485             |         | 1.07 (0.83–1.39)  | 0.589             |         |
| Chronic inflammatory disease | 0.90 (0.52–1.56) | 0.710             |         | 0.74 (0.40–1.40)  | 0.356             |         |
| Anticoagulants            | 0.88 (0.62–1.26)  | 0.493             |         | 0.72 (0.42–1.23)  | 0.228             |         |
| Other thrombocyte inhibitors | 0.82 (0.52–1.29) | 0.398             |         | 1.15 (0.57–2.32)  | 0.705             |         |
| Lipid-lowering agents     | 0.94 (0.71–1.24)  | 0.648             |         | 0.86 (0.63–1.17)  | 0.323             |         |
| ASA                       | 0.68 (0.48–0.95)  | 0.026             |         | 0.93 (0.64–1.34)  | 0.688             |         |
| Valve disease, aortic insufficiency | 23.60 (10.91–51.06) | <0.001            |         | 1.82 (1.37–2.43)  | <0.001            |         |

ASA indicates acetylsalicylic acid; BAV, bicuspid aortic valve; BSA, body surface area; and TAV, tricuspid aortic valve.
Then, ascending aortic cyclooxygenase expression was analyzed in relation to ongoing ASA therapy (yes/no). Interestingly, cyclooxygenase-2 expression was significantly lower in ascending aortas of patients on ASA therapy compared with individuals without ASA medication ($P=0.03$) (Figure 3). In nondilated aortas, however, there was no difference in aortic cyclooxygenase-2 expression between ASA-treated and individuals without ASA ($P=0.42$) (Figure 3), which is in line with the inflammatory induction of cyclooxygenase-2 expression. Of note, there was no correlation between cyclooxygenase-2 gene expression and age (Pearson correlation 0.050, $P=0.751$, Figure S1). Similarly, patients with and without ASA treatment did not differ in variables included in the multivariable model (age, body surface area, known vascular disease valve disease and lipid-lowering agents, Table S1). Moreover, cyclooxygenase-2 expression correlated positively with several macrophage markers, such as CD163, CD14, and CD86, as well as macrophage-related inflammatory cytokines (ie, IL-1β, IL-6, and IL-8 in dilated TAV aortas (Figure 4). There was no difference in cyclooxygenase-1 expression in relation to ASA therapy.

Figure 2. COX expression and ascending aortic dimensions. Aortic medial cyclooxygenase-1 (COX-1) (A) and COX-2 (B) expression plotted against ascending aortic diameter (mm, Y-axis). n=43 patients with tricuspid aortic valve.
with cyclooxygenase-2 expression in nondilated aorta and ASA. Interestingly, ASA therapy was not associated with any protective effect of ASA and the platelet inhibitor clopidogrel may be associated with improved outcomes in AAA, only ASA correlated with improved outcomes in thoracic aortic aneurysms.6

There are limitations to the present study. Surgical cohorts are associated with a selection bias, which may

DISCUSSION
The present study shows an inverse association between preoperative ASA treatment and ascending aortic dilatation at the time of aortic valve surgery in patients with TAV, but not BAV. Gene expression analysis showed a positive correlation between cyclooxygenase-1 and cyclooxygenase-2 and maximum aortic diameter in TAV, and a lower expression of cyclooxygenase-2 in dilated aortic tissue from patients treated with ASA. Interestingly, ASA therapy was not associated with cyclooxygenase-2 expression in nondilated aortas, indicative of an inflammation-induced association. Indeed, as expected, cyclooxygenase-2 expression correlated positively with the expression of proinflammatory cytokines and markers of innate immune cells in the ascending aortic wall. Together these findings are consistent with the hypothesis that ASA treatment may attenuate ascending aortic aneurysmal growth and expansion in patients with TAV, and that this may be mediated via inhibition of cyclooxygenase-2-inflammatory actions. In addition, the study further supports the involvement of inflammation in the pathogenesis of TAV-associated AscAA but not BAV-associated AscAA, which is in line with previous findings.16

Reports on ASA treatment on the outcome of established AscAA and AAAs have shown conflicting results,6,17,18 and its use is not supported by current guidelines.15,19 However, low-dose ASA has previously been associated with a reduced expansion rate of medium-sized AAA,4 and in a recent large systematic review and meta-analysis,5 low-dose ASA treatment was associated with a reduced risk of growth of unruptured intracranial aneurysm, irrespective of treatment duration and frequency. This highlights a potential role of ASA therapy in delaying aneurysmal growth. Moreover, several studies using animal models of aortic aneurysms show potential for the protective effect of ASA on aneurysmal development has thus been proposed to be via cyclooxygenase inhibitory actions. Specifically, ASA inhibits both cyclooxygenase-1 and cyclooxygenase-2 expression,20 thus affecting both platelet aggregation and inflammation. Particularly, cyclooxygenase-2 and prostaglandins have been implicated in AAA progression,9–11 and targeting this pathway attenuates aortic aneurysms in animal models,7,12,13 ostensibly by decreasing aortic inflammation and matrix metalloproteinase activity.21 Here, we show that ascending aortic cyclooxygenase-2 expression levels were inversely associated with ASA therapy in dilated aortic tissue, which is in line with the inhibitory effect of ASA on cyclooxygenase-2 mRNA synthesis.22

While cyclooxygenase-1 is constitutively expressed in most tissues,23 cyclooxygenase-2 expression is undetectable under physiological conditions but induced, predominantly in immune cells, during inflammatory conditions.24 In accordance with this, the inverse correlation of ASA therapy and cyclooxygenase-2 expression could not be observed in nondilated aortas where low-grade inflammation is mainly absent. Moreover, some studies also suggest an alternative protective mechanism of ASA beyond its anti-inflammatory actions, where cyclooxygenase-2 inhibition prevents detrimental effects of prostaglandins on smooth muscle cell survival.9 Lastly, ASA has also been shown to downregulate vascular endothelial growth factor expression in myeloma cells,25 independently of cyclooxygenase inhibition.

Platelet inhibition alone may attenuate AAA and decrease inflammation in animal models,26 but AscAAs are rarely associated with thrombus formation, and selective cyclooxygenase-1 inhibition is ineffective in animal models of aortic aneurysms.11,15 This suggests that any protective effect of ASA in AscAA is unlikely to be mediated by cyclooxygenase-1, but rather is being mediated by effects on cyclooxygenase-2. Indeed, while both ASA and the platelet inhibitor clopidogrel may be associated with improved outcomes in AAA, only ASA correlated with improved outcomes in thoracic aortic aneurysms.6
affect the generalizability of the results. Moreover, the cross-sectional design of the study also leaves the specific relation between ASA therapy and AscAA growth rate to be further elucidated. In addition, the potential influence of other unknown residual confounders cannot be ruled out. However, the study covers one of the largest cohorts of patients undergoing aortic valve and/or ascending aortic surgery, and includes comprehensive data on comorbidities, medications, aortic dimensions, and cardiovascular risk factors. Findings are also supported by gene expression data from a large number of patients.

CONCLUSIONS
There is an inverse association between preoperative ASA treatment and TAV-associated AscAA in patients undergoing cardiac surgery, accompanied by a decreased aortic expression of cyclooxygenase-2 and inflammatory markers in ASA-treated individuals. These findings are consistent with the hypothesis that ASA treatment may attenuate ascending aortic aneurysmal growth, possibly mediated via inhibition of cyclooxygenase-2-mediated vascular inflammation, which could delay or possibly even mitigate the need for aortic replacement in select patients. Further studies explicitly evaluating the potential mediation by cyclooxygenase-2 inhibition are needed, however. ASA is widely used and rarely harmful and could thus be an appealing preventive measure in patients at risk of AscAA who do not meet criteria for valve or aortic surgery (ie, patients with TAV and mild-to-moderate aortic stenosis or aortic insufficiency with mild aortic dilatation, <4.5 cm).

Figure 4. Gene expression correlation matrix.
Cyclooxygenase-2 gene expression in relation to expression levels of inflammatory cytokines (IL1B, IL6, IL8), and macrophage markers (CD14, CD86, CD163) in dilated ascending aortic media of patients with TAV (n=20). Significant Pearson correlation values are presented. IL indicates interleukin; and TAV, tricuspid aortic valve.
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Affiliations
Section of Cardiothoracic Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden (C.G., D.F., F.B., C.O., A.F.); and Cardiovascular Medicine Unit, Center for Molecular Medicine, Department of Medicine, Karolinska Institutet, Stockholm, Karolinska University Hospital, Solna, Sweden (H.M.B.).

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Disclosures
None.

Supplemental Material
Table S1
Figures S1–S3

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|                               | without ASA | with ASA | P-value |
|-------------------------------|-------------|----------|---------|
| Age, y (SD)                   | 64.2 (13.4) | 67.5 (14.1) | 0.444   |
| BSA, m² (SD)                  | 2.0 (0.21)  | 1.9 (0.23)  | 0.261   |
| Known vascular disease, n (%) | 3 (9.7)     | 3 (25)     | 0.325*  |
| Lipid-lowering agents, n (%)  | 6 (19.4)    | 1 (8.3)    | 0.652*  |
| Valve disease (aortic regurgitation), n (%) | 9 (34.6) | 5 (41.7) | 0.728*  |

*Fishers exact test.
Figure S1. Correlation COX-2 gene expression and age. Pearson correlation coefficient 0.050, P=0.751
Figure S2. COX-1 expression and ASA treatment in patients with TAV. COX-1 expression in dilated and non-dilated ascending aortic tissue in TAV patients with (+) and without (-) ASA treatment. N=20 TAV patients with dilatation (-ASA n=15, +ASA n=5), and n=23 TAV patients without dilatation (-ASA n=17, +ASA n=6). Differences were evaluated using unpaired t-test with Welch’s correction.
Figure S3. COX-2 expression and ASA treatment in patients with BAV. COX-2 expression in dilated and non-dilated ascending aortic tissue in BAV patients with (+) and without (-) ASA treatment. N=44 BAV patients with dilatation (-ASA n=35, +ASA n=9), and n=31 BAV patients without dilatation (-ASA n=27, +ASA n=4). Differences were evaluated using unpaired t-test with Welch’s correction.