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Defective NOTCH signalling drives smooth muscle cell death and differentiation in bicuspid aortic valve aortopathy

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Key Question

Are changes in ascending aortic smooth muscle cell NOTCH signalling associated with bicuspid aortic valve (BAV) aortopathy?

Key Findings

NOTCH signalling is associated with changes in BAV smooth muscle cell differentiation and apoptosis gene expression.

Take-home Message

NOTCH signalling in BAV aortic smooth muscle cells is implicated in aortopathy and may be a therapeutic target for preventing apoptosis.
Abstract

Objectives

Bicuspid aortic valve disease is common and is associated with ascending aortic aneurysm. Vascular smooth muscle cell apoptosis is characteristic of the ascending aorta of bicuspid patients and NOTCH1 gene mutations have also been linked to the disease. NOTCH signalling is a fundamental cell signalling pathway, which dictates cell fate decisions including apoptosis. Our objective was to elucidate the role of NOTCH signalling in vascular smooth muscle cell apoptosis and differentiation in bicuspid aortopathy.

Methods

Ascending aortic biopsies were obtained from 19 bicuspid and 12 tricuspid aortic valve patients and were sub-classified into 4 groups according to maximum ascending aortic diameter (aneurysmal=≥45mm). Apoptotic vascular smooth muscle cells were counted by light microscopy using TUNEL assay. Gene expression of key regulators of NOTCH signalling (NOTCH1 and HES1), apoptosis (BAX and BCL-2) and vascular smooth muscle cell differentiation (MYH11, CNN1 and MYH10) were quantified using quantitative real-time PCR. Primary vascular smooth muscle cells were cultured from 2 tricuspid aortic valve and 2 bicuspid aortic valve patients, NOTCH signalling was inhibited with DAPT and gene expression quantified.

Results

Apoptotic cell count was significantly higher in BAV patients (3.2 cells/50,000μm² versus 1.1 cells/50,000μm²; \(p=0.033\)). There was a trend towards lower apoptotic cell count in aneurysmal versus non-aneurysmal tricuspid and bicuspid groups and an increased ratio of pro-apoptotic gene expression, which was not statistically significant. This was associated with a 2.8-fold increase in contractile gene expression \(\left(p=0.026\right)\) and a 2.0-fold increase in NOTCH signalling gene expression in bicuspid versus tricuspid aortic valve patients \(\left(p=0.022\right)\). NOTCH inhibition in cultured vascular
smooth muscle cells induced a similar pattern of increased pro-apoptotic and pro-contractile gene expression.

Conclusions

This preliminary study suggests that NOTCH activation in non-aneurysmal bicuspid aortas may underlie aortopathy by influencing vascular smooth muscle cell apoptosis and differentiation. NOTCH signalling manipulation may provide a therapeutic target for preventing aneurysm in bicuspid patients. Further studies with larger sample sizes are needed to substantiate the present findings.

Keywords

Bicuspid aortic valve, ascending aortic aneurysm, vascular smooth muscle cell, NOTCH signalling, apoptosis, cell differentiation

Introduction

Bicuspid aortic valve (BAV) disease is the most common congenital cardiac anomaly affecting between 1 – 2% of the population with a male predominance of 3:1[1-3]. It results when only two of the three aortic valve leaflets form and is associated with accelerated aortic valve degeneration and need for valve replacement[4]. BAV is also a major risk factor for ascending aortic aneurysm, which affects approximately 50% of patients during their lifetime[5, 6]. Left undetected, aneurysms may rupture or dissect which is frequently fatal. Despite a growing research interest, the mechanisms underlying this association remain to be elucidated.

Microscopic examination of the BAV ascending aortic media reveals medial necrosis, fibrillin degradation, elastin fragmentation and vascular smooth muscle cell (VSMC) apoptosis[7]. VSMC apoptosis is seen in BAV aortas before aortic dilation occurs, suggesting that a pre-existing genetic defect may exist[8]. One of the few genetic mutations identified in BAV populations are those in the
human gene Notch homolog 1, translocation-associated (Drosophila), also known as NOTCH1, an evolutionarily-conserved cell signalling mechanism that dictates cell fate decisions[9, 10]. The pathway is central to the coordination of neural crest cell migration during cardiac embryogenesis. These cells go on to populate the developing ascending aorta as primitive VSMCs[11]. NOTCH signalling is also implicated in apoptosis inhibition[12] and promotion of the contractile VSMC phenotype[13]. Recently, NOTCH signalling in aortic valve endothelial cells has been demonstrated to differ in BAV and TAV patients, and may induce accelerated valve calcification in BAV patients[14].

Currently, there is little evidence for defective NOTCH signalling in BAV aortopathy, and a role in VSMC apoptosis and differentiation remains to be elucidated[15]. Given the central role of NOTCH signalling in cell fate decisions, and its implication in BAV disease, we hypothesised that changes in NOTCH signalling may underlie increased VSMC apoptosis in BAV aortopathy[15]. Furthermore, the influence of NOTCH signalling on cellular differentiation may underlie the failure of VSMCs to respond to and repair the degenerated ECM, which is characteristic of BAV aortopathy[7]. This preliminary study investigates the impact of NOTCH signalling on VSMC apoptosis, apoptotic gene expression and VSMC differentiation gene expression in the context of BAV aortopathy, using both aortic tissue and VSMC culture models. We hypothesise that changes NOTCH signalling may underlie increased vascular smooth muscle cell apoptosis and differentiation in BAV aortopathy, and thus represent a key pathological pathway.

Materials and methods

Patient selection

Ethical approval was granted by Hampshire B NRES committee south central (REC Ref: 11/SC/0258) to collect ascending aortic wall biopsies from patients aged 18-80 years undergoing aortic valve replacement and/or ascending aortic replacement at University Hospital Southampton (Southampton, UK). Written informed consent was provided by study participants who were approached on a
consecutive basis. Biopsies were taken from the anterior aspect of the aorta from the edge of the aortotomy line. Exclusion criteria included mitral valve disease (greater than mild), atherosclerosis of the ascending aorta, infective endocarditis and known genetic conditions (e.g. Marfan syndrome).

Maximum ascending aortic diameter was obtained on perioperative transoesophageal echocardiography and aneurysm was defined as diameter ≥45mm as per the European Society of Cardiology/European Association for Cardio-Thoracic Surgery guidelines[16]. Aortic valve morphology was defined intraoperatively by the operating surgeon and confirmed by a second surgeon as either bicuspid (n=19) or tricuspid (n=12). Subsequently patients were divided into 4 groups; bicuspid aortic valve with dilated/aneurysmal aorta (BD) or undilated/non-aneurysmal aorta (BU) and tricuspid aortic valve with dilated/aneurysmal aorta (TD) or undilated/non-aneurysmal aorta (TU). All morphological assessments concurred with pre-operative TOE images, which were reviewed by a consultant cardiologist. Due to the limited time scale for recruitment, the maximum possible number of patients were enrolled. More BAV patients underwent surgery during the study period, therefore study group numbers differed. Mean age was 61.3 (±11.4) and 65% of the patients (n=20) were male. Ascending aortic aneurysm was present in 10 of 19 BAV and 5 of 12 TAV patients (Table 1).

Quantification of apoptotic VSMCs in the aortic media

Aortic biopsies were snap-frozen in Optimum Cutting Temperature (OCT; Agar Scientific, UK) medium, sectioned at 7µm and mounted on microscopy slides. The TACS TdT In Situ Apoptosis Detection Kit (Trivigen, USA) was used to indicate cell apoptosis according to the standard protocol (Supplementary File 1). Additional control measures included a positive control using a nuclease step (creating double-stranded DNA breaks seen in apoptotic cells) and a negative control which excluded the labelling mix. All samples were stained in duplicate. Each slide was imaged using a dotSlide Virtual Slide System (Olympus, UK). Ten subfields (50,000µm²) were scanned at 40x magnification and a macro for Fiji-ImageJ image analysis software[17] was written to count viable and apoptotic cells. Apoptotic index was calculated:
**Apoptotic Index** = \( \frac{\text{Number of apoptotic nuclei}}{\text{Total number of nuclei}} \times 100 \)

Expression of NOTCH signalling, apoptosis and VSMC differentiation genes in aortic tissue

**RNA extraction**

Snap-frozen aortic samples were crushed on liquid nitrogen prior to RNA extraction as detailed previously by our group using the spin column-based Qiagen RNeasy Fibrous kit (Qiagen, USA) as per protocol[18]. RNA yield and quality were confirmed using a NanoDrop spectrophotometer (Thermo-Fisher Scientific, UK) and gel electrophoresis. RNA integrity was assured using the Agilent Bioanalyser (Agilent Technologies, USA) with mean RIN values of 8.8 (±1.1). RNA was reverse transcribed using GoScript Reverse Transcription Kit (Promega, UK).

**Quantitative real-time PCR (qRT-PCR)**

Primer sequences for the genes of interest can be found in Supplementary Table S1. Gene expression was measured by qRT-PCR (Supplementary File 2).

**Inhibition of NOTCH signalling in VSMCs**

**VSMC culture**

Primary VSMCs were raised from four of the aortic explants used in the aortic tissue experiments (n=4; Table 2). A detailed description of the method is found in Supplementary File 3. VSMCs were seen to grow out from aortic explants in a characteristic ‘hills and valleys’ pattern and demonstrated immunofluorescence for \( \alpha \)-smooth muscle actin.

**Inhibition of NOTCH signalling**

N-[N-(3,5-Difluorophenacyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) is a \( \gamma \)-secretase inhibitor and an indirect inhibitor of NOTCH signalling (Supplementary File 4)[19]. DAPT
concentration and time points were optimised in preliminary experiments. DAPT (dissolved in DMSO) at 1μM in was used together with DMSO/medium only control. Culture wells were seeded with 100,000 cells and performed in duplicate. Cells were incubated for 48 hours at 37°C with 5% CO₂ prior to adding DAPT solutions. Baseline cultures were harvested and experimental solutions were added. Further harvests were performed at 6 and 12 hours. Relative gene expression of NOTCH1, HES1, BAX, BCL-2, MYH11, CNN1 and MYH10 were calculated using qRT-PCR as described above. SDHA and YWAZ were identified as stable reference genes (unpublished data).

Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics package (v24) and GraphPad Prism (v7). One TAV patient and 3 BAV patients had inadequate biopsy mass to perform cell counting and 2 BAV patients had inadequate tissue to perform gene expression. Therefore, cell counts were performed on 11 TAV and 16 BAV samples and gene expression was performed on 12 TAV and 17 BAV patients. P-values for statistical significance were calculated using t-test and analysis of variance (ANOVA) for continuous variables, and Chi-squared test for categorical variables where Yates’ correction was performed if minimum counts were not met. One-way ANOVA was used for aortic gene expression data and two-way ANOVA with Tukey post-hoc test was used for VSMC gene expression data. Linear regression excluded any interaction between valve morphology and aortic diameter such that each could be compared independently. Normality testing was performed on all outcome variables using visual assessment and Shapiro-Wilk Test. Logarithmic transformation was performed prior to statistical testing where outcome variables were not normally distributed, but are presented untransformed. P<0.05 was considered statistically significant. Gene expression values are in arbitrary units relative to reference genes and conveyed as fold-changes. For the VSMC experiments, fold-change in gene expression compared to baseline is quoted. The maximum possible number of samples were obtained within the timescale of the project. However, a retrospective power calculation based on α (type I error) of 0.05 and power of 0.8 suggested a sample size of 20 per group.
Results

Quantification of apoptotic VSMCs in the aortic media

Apoptotic cell count was significantly higher in BAV versus TAV patients (3.2 cells/50,000µm² versus 1.1 cells/50,000µm²; p=0.033), in the absence of any significant difference in viable cell count (23.0 cells/50,000µm² versus 19.0 cells/50,000µm²; p=0.442; Figure 1A and B). Although apoptotic index was 1.5-fold higher in the BAV group, this was not statistically significant (p=0.299; Figure 1C). There was no significant difference in either viable or apoptotic cell count between TAV and BAV patients with aneurysmal versus non-aneurysmal aortas (Figure 1D and E). However, there was a trend towards decreased apoptotic cell count in aneurysmal versus non-aneurysmal TAV and BAV aortas (0.3 cells/50,000µm² versus 1.6 cells/50,000µm² and 2.2 cells/50,000µm² versus 4.4 cells/50,000µm² respectively; p=0.087). Apoptotic index followed a similar trend between aneurysmal and non-aneurysmal aortas but this was not statistically significant (Figure 1F). A comparison between aneurysm and non-aneurysm groups are seen in Supplementary Figure S1.

Expression of NOTCH signalling, apoptosis and VSMC differentiation genes in aortic tissue

Apoptotic gene expression

Expression differences in pro-apoptotic BAX and anti-apoptotic BCL-2 genes between BAV and TAV patients with non-aneurysmal and aneurysmal aortas were calculated. A ratio between BAX and BCL-2 (apoptotic factor) was calculated as a measure of tendency towards apoptosis as has been used previously[20]. BAX expression was significantly higher in aneurysmal TAV patients compared to non-aneurysmal TAV patients (2.3-fold, p=0.001), non-aneurysmal BAV patients (1.7-fold, p=0.011) and aneurysmal BAV patients, (1.5-fold, p=0.025; Figure 2A). BCL-2 expression was lower in aneurysmal versus non-aneurysmal aortas (0.9-fold for both TAV and BAV), but this was not
statistically significant (Figure 2B). Apoptotic factor (BAX:BCL-2 ratio) reflected these observations (Figure 2C).

VSMC differentiation gene expression

Given the paradoxical association between apoptotic gene expression and apoptotic cell count in aneurysmal versus non-aneurysmal aortas, it was hypothesised that differences in VSMC differentiation may occur in parallel. Expression of contractile phenotype genes MYH11 and CNN1, together with the synthetic phenotype gene MYH10 were calculated[21]. Expression of all three VSMC differentiation genes tended to be higher in aneurysmal BAV aortas, and lower in aneurysmal TAV aortas compared to their non-aneurysmal equivalents, but these were not statistically significant (Figure 3A-C). Contractile factor (MYH11xCNN1:MYH10) was significantly higher by 2.8-fold in aneurysmal BAV versus aneurysmal TAV patients (p=0.026; Figure 3D). Furthermore, contractile factor was 1.3-fold higher in aneurysmal versus non-aneurysmal BAV aortas and 0.5-fold lower in aneurysmal versus non-aneurysmal TAV aortas, but this was not found to be statistically significant.

NOTCH signalling gene expression

It was hypothesised that the differences in apoptosis and differentiation gene expression may be linked to NOTCH signalling activation. To elucidate this, the expression of NOTCH1 and its downstream target HES1 were calculated. There was no significant difference in NOTCH1 expression between BAV and TAV patients of differing aortic dimensions (Figure 4A). However, HES1 expression was significantly higher in BAV patients with non-aneurysmal aortas by 2.0-fold versus their TAV counterparts (p=0.022; Figure 4B). HES1 expression was 0.7-fold lower in the aneurysmal BAV group versus the non-aneurysmal group but this was not found to be statistically significant. A reciprocal trend towards increased HES1 expression by 2.0-fold in aneurysmal versus non-aneurysmal TAV patients was also seen, but again this was not found to be statistically significant.
Inhibition of NOTCH signalling in VSMCs

To investigate whether gene expression changes could be reproduced in-vitro, NOTCH signalling was inhibited in primary VSMC cultures with DAPT. At 6 and 12 hours, HES1 expression was significantly reduced by 0.4-fold in the BAV DAPT treated cells versus control (p<0.05), an effect that was not replicated in the TAV DAPT treated cells (Figure 5A). Apoptotic factor demonstrated significant decreases from baseline in both TAV control cells by 0.6-fold (p<0.05) and TAV DAPT cells by 0.4-fold (p<0.01) at 6 hours (Figure 5B). A similar trend was seen in the BAV cells at 6 hours, but this was not found to be statistically significant. Nevertheless, NOTCH inhibition with DAPT attenuated this decrease compared to the BAV control. A similar pattern was seen at 12 hours, however apoptotic factor increased in the TAV DAPT cells by 1.1-fold compared to baseline and decreased in the TAV control by 0.6-fold compared to baseline, but this was not significant. Contractile factor was significantly reduced by 0.5-fold compared to baseline in the BAV control cells at 12 hours (p<0.05), and there was a trend towards reduction in the BAV DAPT cells by 0.7-fold although this was not statistically significant (Figure 5C). This trend was reflected in the BAV control and BAV DAPT cells at 6 hours, but again was not statistically significant.

Discussion

Increased VSMC apoptosis in the ascending aorta of patients with BAV disease is well documented, but the underlying mechanisms, particularly the contribution of VSMC differentiation state and NOTCH signalling has yet to be elucidated. This study demonstrated an inverse relationship between observed VSMC apoptosis and apoptotic gene expression in BAV aortopathy, which was associated with contractile VSMC differentiation. NOTCH signalling activation demonstrated a similar association, which was confirmed in-vitro where NOTCH inhibition promoted the contractile phenotype and pro-apoptotic gene expression. These preliminary findings suggest that defective NOTCH signalling may underlie the pathophysiology of BAV aortopathy and represents an area for further research.
Increased VSMC apoptosis is a hallmark of BAV aortopathy but the molecular mechanisms are poorly understood[22]. The present study demonstrated that in aneurysmal aortae, the number of cells undergoing apoptosis tends to decrease in both BAV and TAV patients, which is consistent with previous reports[22]. However, a paradoxical increase in BAX:BCL-2 ratio was also seen implying that VSMCs may develop resistance to apoptosis. Previous studies suggest that the synthetic VSMC phenotype may confer resistance to apoptosis[23]. The present results support this, demonstrating lower contractile gene expression associated with higher apoptotic gene expression and lower apoptotic cell count in the TAV group. Conversely, VSMCs in aneurysmal BAV aortas demonstrated higher contractile gene expression, suggesting increased sensitivity to apoptosis which may contribute to aortopathy.

NOTCH may play a role in any such changes in VSMC differentiation, however this is disputed [24, 25]. NOTCH1 is an important gene in BAV disease and our hypothesis was that changes in NOTCH signalling may contribute to BAV aortopathy. Significantly increased HES1 expression (a surrogate marker of NOTCH activation) in non-aneurysmal BAV aortas versus non-aneurysmal TAV aortas was demonstrated with a paradoxical decrease in BAV and increase in TAV with aneurysm. A recent study by Balistreri et al. similarly concluded that NOTCH activation increases in aneurysmal versus non-aneurysmal TAV aortas, and aneurysmal TAV versus aneurysmal BAV aortas[26]. One possible explanation is that infiltrating inflammatory cells (characteristic of TAV aneurysms) expressing NOTCH ligands increase VSMC NOTCH activation[27]. Similarly, inhibition of NOTCH signalling in abdominal aortic aneurysms (also mediated by inflammation) reduces aortic diameter in a mouse model[28].

Another important observation from the present study was that inhibiting NOTCH signalling with DAPT significantly reduced HES1 expression in BAV VSMCs, but did not affect HES1 expression in TAV VSMCs. As is suggested by our results, a defect in activation of NOTCH signalling in BAV VSMCs may be present, for example a difference in the activity of γ-secretase or its affinity for DAPT. Alternatively, there may be reduced expression of NOTCH ligands in BAV VSMCs as previously demonstrated by Sciacca et al.[29]. Reduced ligand expression in culture may lead to
reduced NOTCH receptor activation and $HES1$ expression, and a possible compensatory upregulation in NOTCH receptor transcription.

A definitive conclusion in this preliminary study is difficult, but the results strongly suggest that inhibition of NOTCH signalling in BAV VSMCs promotes the contractile VSMC phenotype and pro-apoptotic gene expression. Previous studies have proposed NOTCH signalling activation reduces apoptotic drive and promotes the synthetic phenotype in VSMCs[12, 30]. We postulate that NOTCH signalling may be central to BAV aortopathy by directly increasing apoptosis and promoting the contractile VSMC phenotype, which further sensitises VSMCs to apoptosis.

This study presents a number of limitations. The limited sample size may have resulted in the lack of statistical significance in some of the comparisons and several hypotheses rely on trends which will require further validation. Differing shear stresses on the aortic wall may affect gene expression. Although samples were taken from the same area, we cannot exclude an effect of shear stresses on gene expression. Furthermore, the data would have been strengthened if validated at the protein level. Any mismatch between transcription and translation could result in significant mRNA changes without a change in the biologically active proteins. An attempt was made to correlate aortic and VSMC gene expression. There appeared to be some congruency between gene expressions according to valve morphology, but this was not statistically significant. Similarly, $HES1$ expression was assumed to correlate with NOTCH activation, however this is an indirect indicator and may be influenced by other pathways. Additionally, using two inhibitors of NOTCH signalling would have greatly strengthened our conclusions. Furthermore, activation of other NOTCH receptors 2, 3 and 4 may be influencing the results, but was not detected by our methodology. Finally, all samples used in the VSMC culture experiments were taken from aneurysmal aortae and therefore may not be representative of findings in non-aneurysmal aortae. Several unsuccessful attempts were made to culture VSMCs from non-aneurysmal samples, likely due to limited tissue mass.

The complex mechanisms underlying BAV aortopathy have afforded extensive research to date, and will undoubtedly continue to be the focus of intense future investigations. This preliminary study has
provided some insight into the role of VSMC apoptosis and differentiation in BAV aortopathy, and described the important interaction of NOTCH signalling within these mechanisms. NOTCH signalling has been identified as an important pathway in BAV disease, and may represent a future therapeutic target for preventing the progression of BAV aortopathy. Further studies are needed to substantiate these findings.

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Funding Statement

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Conflict of interest

None declared.
Table 1 | Characteristics of the study groups (mean ± standard deviation or % rounded to nearest whole number). P-values are given for difference between the groups for each demographic.

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<td>0</td>
</tr>
<tr>
<td>&lt;35%</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
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AAR, ascending aortic replacement; ARR, aortic root replacement; AVR, aortic valve replacement; BD, bicuspid aortic valve, aneurysmal; BU, bicuspid aortic valve, non-aneurysmal; TD, tricuspid aortic valve, aneurysmal; TU, tricuspid aortic valve, non-aneurysmal.

Table 2

Demographics and culture data of the patients used for NOTCH signalling inhibition in VSMCs
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>66</th>
<th>59</th>
<th>63</th>
<th>71</th>
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<tr>
<td>Gender</td>
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<td>Male</td>
<td>Male</td>
<td>Male</td>
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<tr>
<td>Aortic valve morphology</td>
<td>Tricuspid</td>
<td>Bicuspid</td>
<td>Bicuspid</td>
<td>Tricuspid</td>
</tr>
<tr>
<td>Max aortic diameter (mm)</td>
<td>49</td>
<td>54</td>
<td>57</td>
<td>57</td>
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<tr>
<td>Time to ≥80% confluence (days)</td>
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<td>37</td>
<td>31</td>
<td>24</td>
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<tr>
<td>Cell count at first passage</td>
<td>1,005,000</td>
<td>206,400</td>
<td>606,000</td>
<td>808,644</td>
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<tr>
<td>Passage at which cells used</td>
<td>P2</td>
<td>P3</td>
<td>P2</td>
<td>P2</td>
</tr>
</tbody>
</table>

Figure legends

Figure 1

TUNEL cell counts by valve morphology and maximum ascending aortic diameter. A & D: Mean viable cell count/50,000μm². B & E: Mean apoptotic cell count/50,000μm². C & F: Mean apoptotic index. BAV patients with non-aneurysmal (BU; n=7) and aneurysmal (BD; n=9) aortas, and TAV patients with non-aneurysmal (TU; n=7) and aneurysmal (TD; n=4) aortas. Error bars=±1SE. *=p<0.05.

Figure 2

Ascending aortic apoptotic gene expression in BAV and TAV patients by aortic dimension. A: Mean BAX expression; B: Mean BCL-2 expression; D: Mean apoptotic factor (BAX:BCL-2 ratio). BAV patients with non-aneurysmal (BU; n=7) and aneurysmal (BD; n=10) aortas, and TAV patients with non-aneurysmal (TU; n=7) and aneurysmal (TD; n=5) aortas. Expressions relative to reference genes (GAPDH and UBC). Error bars=±1SE. *=p<0.05, **=p<0.01, ***=p<0.001.
Figure 3

Ascending aortic VSMC differentiation gene expression in BAV and TAV patients by aortic dimension. A: Mean MYH11 expression; B: Mean CNN1 expression; C: Mean MYH10 expression; D: Mean contractile factor (MYH11xCNN1:MYH10 ratio). BAV patients with non-aneurysmal (BU; n=7) and aneurysmal (BD; n=10) aortas, and TAV patients with non-aneurysmal (TU; n=7) and aneurysmal (TD; n=5) aortas. Expressions relative to reference genes (GAPDH and UBC). Error bars=±1SE. *=p<0.05.

Figure 4

NOTCH signalling gene expression in the ascending aortas of BAV and TAV patients by aortic dimension. A: Mean NOTCH1 expression; B: Mean HES1 expression. BAV patients with non-aneurysmal (BU; n=7) and aneurysmal (BD; n=10) aortas, and TAV patients with non-aneurysmal (TU; n=7) and aneurysmal (TD; n=5) aortas. Expressions relative to reference genes (GAPDH and UBC). Error bars=±1SE. *=p<0.05.

Figure 5

Fold-change in NOTCH signalling, apoptosis and VSMC differentiation gene expression compared to baseline at 6 and 12 hours following inhibition of NOTCH signalling with DAPT in primary VSMC cultures. A: HES1 gene expression changes. B: Apoptotic factor (BAX:BCL2 ratio) changes. C: Contractile factor (MYH11xCNN1:MYH10 ratio) changes. Expressions relative to reference genes (SDHA and YWHAZ). N=2 in each experimental group. Bars represent mean change in expression compared to baseline at time=0 hours. *= p<0.05 and **=p<0.01.
References


