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Upregulation of the *high mobility group AT-hook 2* gene in acute aortic dissection is potentially associated with endothelial-mesenchymal transition

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Summary. *The high mobility group AT-hook 2 (HMGA2)* gene is proposed to regulate the genes involved in the epithelial-mesenchymal transition (EMT). One form of EMT is endothelial-mesenchymal transition (EndMT). We analyzed the expression profile of the *HMGA2* gene in different human aortic diseases.

Aortic specimens were collected from 51 patients, including 19 with acute aortic dissection, 26 with aortic aneurysm, two with Marfan syndrome and four aortic valves. Quantitative real-time polymerase chain reaction was carried out for *HMGA2* and immunohistochemical analyses were performed for *HMGA2*, SNAI1, Vimentin, CD34, MKI-67 and TGFB1. The expression of *let-7d* microRNA, which is assumed to play a role in the regulation of *HMGA2*, was also quantified.

The level of *HMGA2* gene expression was significantly higher in acute aortic dissection compared with all the other samples (193.1 vs. 8.1 fold normalized to calibrator, $P<0.001$). The immunohistochemical investigation showed that HMGA2, SNAI1, and Vimentin proteins were mainly detected in the endothelial cells of the *vasa vasorum*.

The *HMGA2* gene is upregulated in acute aortic dissection. This is the first report describing a link between HMGA2 and acute aortic dissection. The HMGA2, SNAI1 and Vimentin proteins were mainly detected in the endothelium of the *vasa vasorum*. It seems that *HMGA2* overexpression in acute aortic dissection occurs in a let-7d-independent manner and is associated with EndMT of the *vasa vasorum*.

Key words: HMGA2, Acute aortic dissection, EMT/EndMT, Vasa vasorum

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Introduction

The *high mobility group AT-hook 2* (*HMGA2*) gene has the ability to bind to AT-rich DNA sequences and to induce global changes in chromatin structure and thereby regulate transcription (Aravind and Landsman, 1998; Merika and Thanos, 2001). It mediates epithelial-mesenchymal transition (EMT) in response to transforming growth factor beta (TGFB)-induced Smad pathway (Valcourt et al., 2005; Thuault et al., 2006; Zeisberg et al., 2007). Epithelial-mesenchymal transition describes a process that reorganizes epithelial cells into migratory mesenchymal cells (Valcourt et al., 2005). This process is critical to normal embryogenesis and is a defining structural feature of organ development. One form of EMT is endothelial-mesenchymal transition (EndMT), which occurs during the embryonic development of the heart (Zeisberg et al., 2007). Among the modulators of EMT is snail homolog 1 (SNAI1), a transcriptional repressor, which is regulated by *HMGA2* (Thuault et al., 2006). The SNAI1 protein acts as a strong repressor of epithelial-specific genes such as *E-cadherin,* thereby promoting EMT, and it is considered to be a marker of EMT (Peinado et al., 2004; Lee et al.,

2006). During aortic and heart valve maturation SNAI1 is required for TGFB-induced EndMT of embryonic stem cells (Kokudo et al., 2008).

Normally, the level of *HMGA2* expression is very high during embryonic development, whereas it is low, or even undetectable, in differentiated adult cells (Chiappetta et al., 1996; Rogalla et al., 1996). The expression of *HMGA2* is controlled by the microRNAs of the let-7 family, which target its 3'UTR (Lee and Dutta, 2007). Reactivation of *HMGA2* due to cytogenetically detectable aberrations of chromosomal region 12q13-15 is found in a variety of tumors of mesenchymal origin (Abe et al., 2003; Borrmann et al., 2003; Fusco and Fedele, 2007; Belge et al., 2008). Pathogenetically, re-expression of *HMGA2* has also been implicated in the formation of arteriosclerotic plaques and restenosis (Zhou et al., 1995; Chin et al., 1999; Anand and Chada, 2000). However, the involvement of *HMGA2* in acute aortic dissection (AAD) is unknown. Acute aortic dissection is a life-threatening disease with high morbidity and mortality rates, and is generally an unpredictable event (Wheat, 1987; Abbara et al., 2007; Isselbacher, 2007). People commonly at risk of this disease include those with connective tissue disorders such as Marfan syndrome, Ehlers-Danlos syndrome and Erdheim Gsell medial necrosis, but also bicuspid aortic valve. Bicuspid aortic valve is the most common form of congenital heart disease with a frequency of concomitant aortic dilatation of 45-50% (Dietz et al., 1994; Silverman et al., 1995; Beroukhim et al., 2006). Indeed, patients with bicuspid aortic valve are at an increased risk of developing ascending aorta complications and AAD (Januzzi et al., 2004). The pathophysiological mechanism in AAD is most likely multifactorial (Guo et al., 2007; Mohamed et al., 2008).

Since HMGA2 is known as a crucial factor in cardiogenesis, a dysregulation may cause alterations in adult vascular tissues (Monzen et al., 2008). Therefore, we examined *HMGA2* expression in different aortic diseases. Acute aortic dissection patients, who do not suffer from Marfan syndrome, were compared with patients suffering from thoracic aortic aneurysm or Marfan syndrome. We studied the *let-7* miRNA expression in patients with AAD and compared it to the expression of *HMGA2* to reveal a possible dysregulation of *HMGA2* and its inhibitory miRNA. Furthermore the protein levels of *HMGA2*, SNAI1, Vimentin and TGFB were investigated by immunohistochemistry.

Material and methods

Tissue samples

The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all patients. In cases of aortic valve or ascending aorta replacement, diseased aortic tissue was collected during surgery and carefully divided into two parts. One part was immediately snap-frozen in liquid nitrogen and preserved at -80°C to be used later for gene expression and protein analyses. The second part was fixed in 4.5% paraformaldehyde and then embedded in paraffin. The patients' demographics are shown in Table 1.

RNA isolation, reverse transcription, and qRT-PCR for HMGA2 measurement

Total RNA was purified according to the RNeasy® Mini Kit protocol for isolation of total RNA from heart, muscle and skin tissue, including on-column DNaseI digestion and homogenization with TissueLyser (Qiagen, Hilden, Germany). The input amount of tissue was about 5-10 mg. The RNA was quantified and 5 μg RNA was treated with a second DNaseI (6.75 U) digestion for 15 min at room temperature and a cleanup, according to the RNeasy Mini Kit protocol, to remove as much contaminating DNA as possible. Up to 250 ng of total RNA was reverse transcribed with 200 U of M-MLV reverse transcriptase and 150 ng random hexamers according to the manufacturer's instructions (Invitrogen, Karlsruhe, Germany). RT-PCR amplification was performed using the ABI Prism 7300 Sequence Detection System (Applied Biosystems, Darmstadt, Germany). The relative quantification method was used with 18S rRNA as the endogenous control.

Expression analyses of *HMGA2* and 18S *rRNA* were performed in triplicate in a total volume of 20 μl using 2 μl of each cDNA corresponding to 25 ng of total RNA. For the measurement of gene expression the following

Table 1. Patient demographics and clinical characteristics.

Disease	Type A Dissection, N=19	Ascending Aneurysms, N=26	Marfan Syndrome, N=2	Valve Replacement, N=4
Demographic				
Age (years)	59.6±16.4	59.3±13.8	31.5±6.4	54.0±9.2
Gender (male/female)	9m/10f	22m/4f	2m	4m
Body mass index	26.2±4.0	26.1±3.4	25.5±7.8	27.7±5.5
Aortic diameter (mm)	n.a.	53.3±7.1	70.0±14.1	38.0±1.6
Bicuspid aortic valve	N=2	N=14	0	N=3

Absolute values (± SD); n.a.: not available.

sequences were used: *HMGA2*: assay number Hs00171569, Applied Biosystems, with amplicon size of 65bp; 18S rRNA: forward primer: AATTTCACA TAGCCCACTTACATTTACA; reverse primer: TTGATTCTAATAATCCCATGCTTTGA; TaqMan (probe with amplicon size of 65 bp): 6-FAM-ACTGAAGAGTAATCAATCTA-MGB. Due to the higher expression of 18S rRNA the cDNA for these samples was diluted 1:10. The PCR conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C.

RNA isolation, reverse transcription, and qRT-PCR for let-7d measurement

Aortic tissue (80 mg) was homogenized with a TissueLyser (Qiagen, Hilden, Germany) in 1000 μl of TRIzol® Reagent following the manufacturer's instructions (Invitrogen, Karlsruhe, Germany). The RNA was resuspended in 80 μl of nuclease-free water.

Real-time RT PCR was performed in two steps using TaqMan® The MicroRNA assays (Applied Biosystems, Darmstadt, Germany). Twenty nanograms each of miRNA *let-7d* and endogenous control *RNU6B* were reverse transcribed with gene-specific stem-loop primers according to the manufacturer's protocol using the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). In addition, 0.9 μg/μL whole-pooled fetal RNA (Stratagene, Karlsruhe, Germany) was used as an additional control. Real-time PCR was conducted with a 2x Universal Master Mix and 20x TaqMan® assay. MicroRNA assays were purchased as pre-optimized assays using the Applied Biosystems 7300 Real-time PCR System (Applied Biosystems, Darmstadt, Germany). Each reaction was run in triplicate, containing 1.33 μl of cDNA in a final reaction volume of 20 μl. Data were analyzed using the 7300 system software (Applied Biosystems, Darmstadt, Germany) and the linear regression plot for ΔC_t analyses between *let-7d* and *RNU6B* was about 0.1, whereas the *RNU6B* expression was constant in each run by a threshold of 25.0±0.7.

Immunohistochemical analysis and fluorescence double labeling

Immunohistochemical analyses were performed for HMGA2, the EMT markers SNAI1 and Vimentin, TGFB1, the endothelial cell marker CD34 and MKI-67, a nuclear protein associated with cell proliferation. Formalin-fixed and paraffin-embedded tissue sections (4 μm) were deparaffinized, rehydrated and washed with PBS before immunoperoxidase staining. The slides were incubated overnight at 4°C in a humidified chamber with 1:20 rabbit anti-HMGA2 (sc-30223, Santa Cruz Biotechnology, Santa Cruz, California, USA), 1:30 rabbit anti-SNAI1 (sc-28199, Santa Cruz Biotechnology, Santa Cruz, California, USA), 1:200 rabbit anti-Vimentin (HPA001762, Sigma-Aldrich, Munich, Germany), 1:50 mouse anti-CD34 (NCL-END, Leica Biosystems, Newcastle, United Kingdom), 1:100 mouse anti-TGFB1 (ab27969, Abcam, Cambridge, United Kingdom), and 1:400 mouse anti-MKI-67 (M7240, Dako, Glostrup, Denmark). A biotinylated anti-rabbit or anti-mouse link was used as a secondary antibody (for 30 min). The slides were then incubated with an avidin-biotin enzyme label (Vector Laboratories, California, USA) for 30 min and developed with AEC peroxidase substrate (Camon Laborservice GmbH, Wiesbaden, Germany) for 10 min. Finally, the slides were counter-stained with hematoxylin.

For fluorescence double labeling antibodies against Vimentin (rabbit anti Vimentin, Sigma-Aldrich, Munich, Germany) and N-Cadherin (mouse anti N-Cadherin, Abcam, Cambridge, United Kingdom) were used in concentrations of 1:200 and 1:25 respectively. Secondary antibodies with Texas red (Vimentin) (Santa Cruz Biotechnology, Santa Cruz, California, USA) and FITC (N-Cadherin) (Sigma-Aldrich, Munich, Germany) were used. Nucleoli were stained with DAPI.

Statistics

Satistical analysis was performed using Excel/WinSTAT software (R. Fitch software, Bad Krozingen, Germany). Data are presented as mean ± SD. A two-sided Mann-Whitney U-test was performed when continuous data were considered and a two-sided Fischer's exact test was used when relative frequencies were compared. The association between different parameters was analyzed by Pearson's correlation coefficient. Significant differences were assumed at $P \leq 0.05$.

Results

Measurement of HMGA2 gene expression in aortic tissue using qRT-PCR

The levels of *HMGA2* mRNA were quantified using real-time quantitative PCR. Gene expression was measured in the aortic tissue of 19 patients with acute aortic dissection (AAD), 26 patients with thoracic aortic aneurysm (TAA) and two patients suffering from Marfan syndrome (MS); aortic valve tissue of four patients who did not suffer from dissection was also included. Figure 1 demonstrates the log of quantified *HMGA2* expression using the ΔC_t method.

The expression of *HMGA2* in non-dissection aortic disease samples (including two MS, four aortic valves, and 26 TAA) was moderate (Fig. 1A). Samples obtained from patients with TAA featured a mean level of 8.6±9.7-fold expression when compared to the calibrator sample. Samples of aortic tissue from patients with Marfan syndrome showed a mean expression level of 7.9±5.8 fold and mean expression in aortic valve tissue was 5.5±3.9 fold. Differences between these groups were assessed with the Mann-Whitney U-test and no

significant variation was detected. These groups were considered to feature similar *HMGA2* expression and were amalgamated in a group designated "without dissection".

The *HMGA2* expression ranged from 5.2 to 1212.4 fold (mean level = 193.1±272.8) in tissues of AAD patients (Fig 1B). The difference between the AAD samples and the "without dissection" group was highly significant in the Mann-Whitney U-test ($P=1.4*10^{-7}$) but differences between AAD and the non-dissection subgroups were also significant.

The existence of valve malformations, such as the bicuspid aortic valves (BAV), had no significant impact on *HMGA2* expression in TAA patients (BAV vs. TAV: P=0.47). The involvement of age, BMI and aortic diameter was also considered, but none showed a significant correlation with *HMGA2* expression in the different groups (data not shown).

The 19 cases of AAD were composed of 9 male and 10 female individuals. The difference in age between male and female individuals with AAD was found to be significant (mean age male vs female = 50.4±18.0 vs 67.8±11.0 years, P=0.03). The *HMGA2* expression was three-fold higher in female AAD patients compared with male AAD patients (296.0±337.0 vs. 79.0±110.0 fold, P=0.02), (Fig 2). However, *HMGA2* expression was still

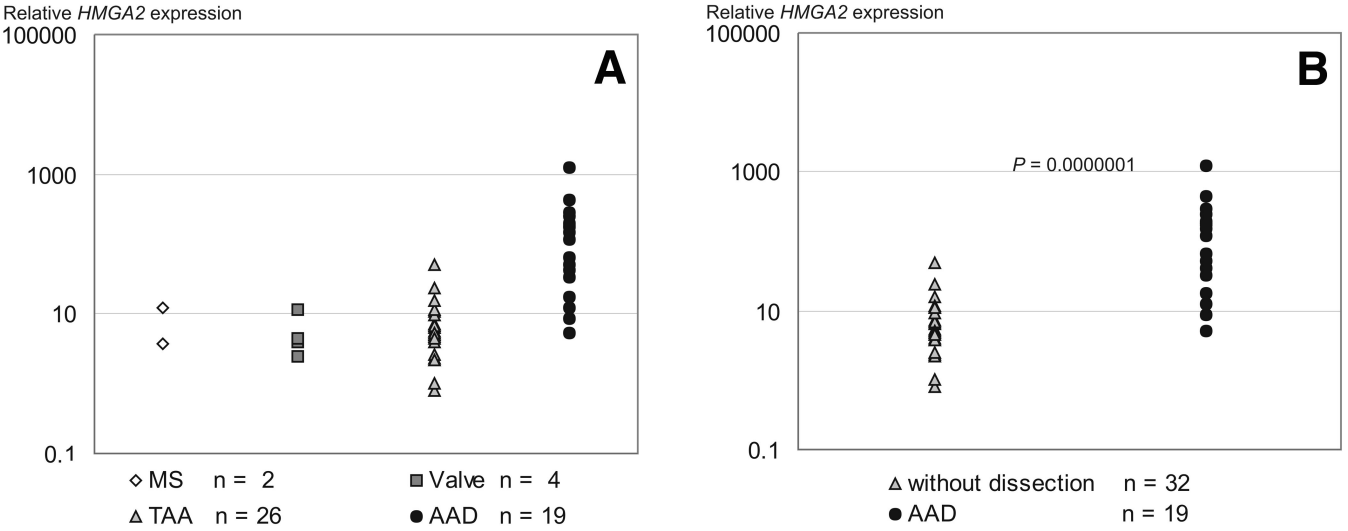

Fig. 1. Logarithmic data of *HMGA2* expression in tissue samples from patients with different aortic diseases. **A.** Comparison of *HMGA2* expression between patients with Marfan syndrome, tissue from aortic valves and patients with thoracic aortic aneurysm. **B.** *HMGA2* expression in different aortic tissues compared to AAD. Samples from Marfan syndrome patients, TAA patients and aortic valve tissue were grouped together and compared with samples obtained from acute aortic dissection, P=0.0000001.

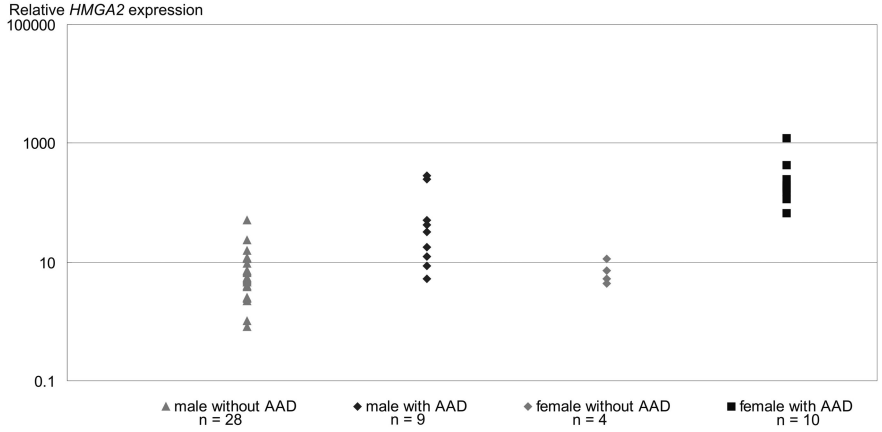

Fig. 2. Gender difference in HMGA2 expression of AAD patients. HMGA2 expression in AAD is three-fold higher in females compared with males (P=0.02). However, these differences in HMGA2 expression were nine-fold higher when we compared females/males with and without dissection (females with AAD vs. females without AAD: P=0.005, males with AAD vs. males without AAD: P=0.0007).

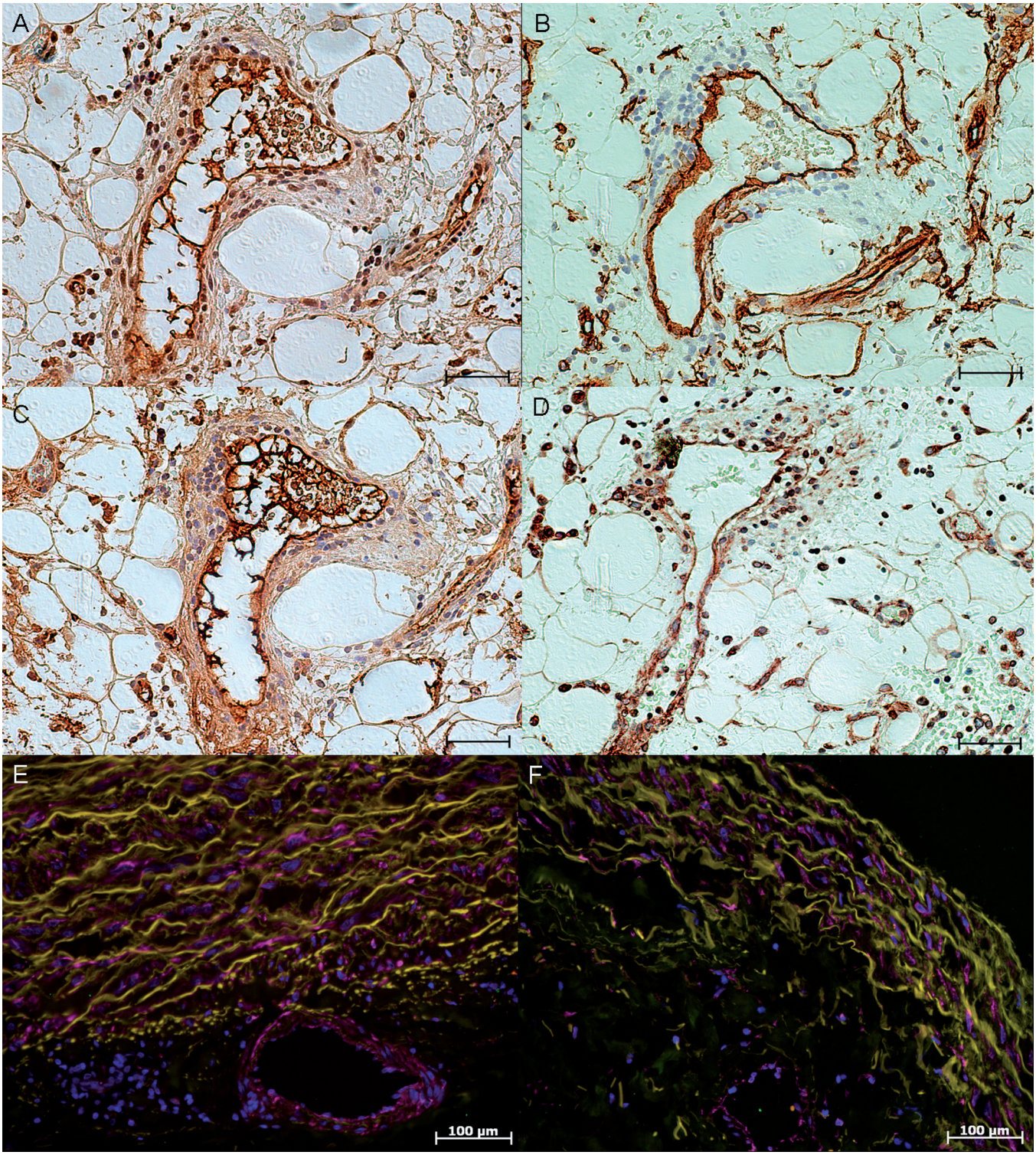

Fig. 3. Immunohistochemical staining and fluorescence double labeling of aortic tissue slides. **A.** HMGA2-positive cells in and around the *vasa vasorum* of an AAD case. **B.** Staining with CD34 reveals the inner vasa vasorum cells to be endothelium. **C.** Immunohistochemistry of the same *vasa vasorum* shows SNAI1 expression in the endothelial cells. **D.** Again the same vasa vasorum, this time the EMT marker Vimentin is found in and around the *vasa vasorum* endothelium. **E and F.** Fluorescence double labeling of two different cases. Purple staining shows the presence of Vimentin, which can also be detected in cells surrounding the *vasa vasorum.* Green/yellow staining indicates autofluorescence. Tissue slides in A, B, C and D were counter stained with hematoxylin. Scale bars: 100 μm.

nine-fold higher when we compared female or male AAD patients with their corresponding group without dissection (female with AAD vs. female without AAD: P=0.005, male with AAD vs. male without AAD: P=0.0007), (Fig 2).

Immunohistochemical analysis and fluorescence double labeling

Immunohistochemical analysis was performed to determine the localization of HMGA2, SNAI1, Vimentin, CD34, TGFB1 and MKI-67 proteins within the aorta. Tissue slides of AAD patients (n=11) and slides of tissue without AAD (n=11) were incubated with the antibodies (Fig. 3). For Vimentin staining tissue slides of 19 AAD patients and 10 patients without AAD were used. Fluorescence double labeling was carried out for the EMT markers Vimentin and N-Cadherin.

HMGA2-positive cells were found in the endothelium and abundantly in the cells surrounding the vasa vasorum (Fig. 3A). These cells were also proven to be endothelial by immunohistochemistry with the endothelial marker CD34 (Fig. 3B), but the EndMT marker SNAI1 was found here as well (Fig. 3C). Vimentin was used as a second marker for EndMT. It was also primarily located in the *vasa vasorum* endothelium and cells surrounding these endothelium (Fig. 3D). Fluorescence double labeling was only positive for Vimentin but negative for N-Cadherin (Fig. 3E,F). Again Vimentin was found in the endothelium of the *vasa vasorum*.

The TGFB1-positive cells were detected in all aortic layers but the staining was too diffuse for interpretation. No increased cell proliferation was detected using the

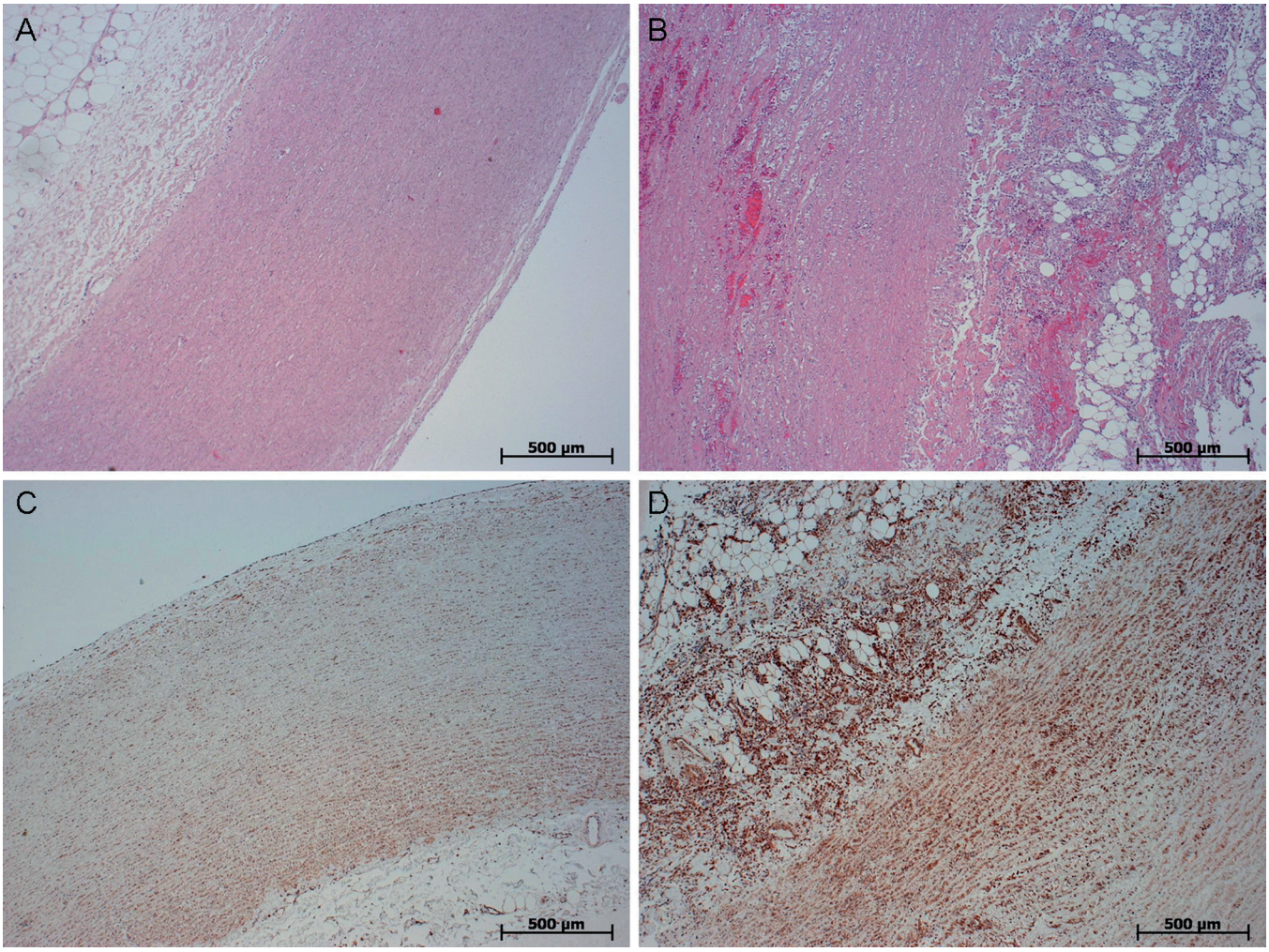

Fig. 4. Hematoxylin-eosin staining of AAD tissues and Vimentin immunostaining of comparable areas. **A and B.** H&E staining of AAD aortic tissue from two different patients. **C and D.** Detection of Vimentin in areas comparable to A and B. The vasa vasorum in the adventitia shows increased Vimentin abundance. Scale bars: 500 μm.

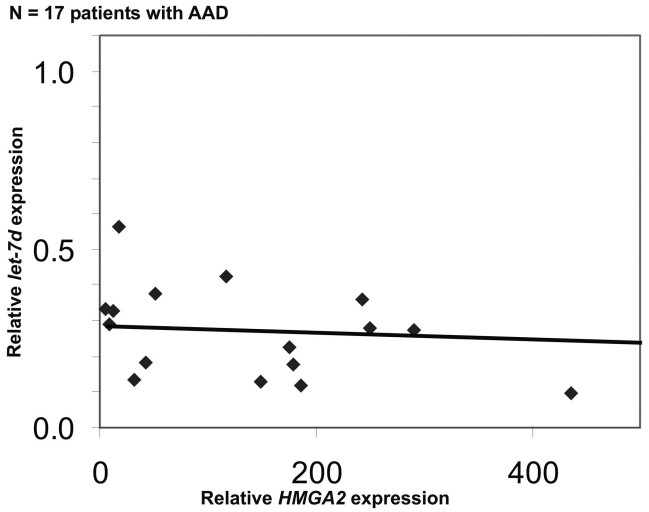

Fig. 5. Relative *HMGA2* and *let-7d* expression. Comparison of quantified relative *HMGA2* and *let-7d* expression of 17 patients with AAD revealed no significant differences. The correlation between the two sample sets (*let-7d* and *HMGA2* expression of 17 AAD patients) was very poor at R^2=0.04, P=0.86

human MKI-67 antibody (data not shown).

The HMGA2 and SNAI1 proteins were found in 7 out of 11 cases originating from AAD patients, whereas only 2 out of 11 cases from other aortic diseases, including aortic aneurysm, were positive (P=0.08). Vimentin was detected in 10 out of 19 AAD slides and in 2 out of 10 slides from control patients (P=0.1). The difference between these findings may improve in a greater sample number.

Figure 4 demonstrates Hematoxylin-eosin staining of AAD tissue and Vimentin immunostaining of the same area. Vimentin was observed in the *media* and *adventitia* and was most abundant in the *vasa vasorum*.

Detection of let-7d in aortic tissue of AAD

The level of the *let-7* variant *d* was quantified in 17 AAD tissue samples to study whether *HMGA2* expression in AAD is regulated by this miRNA. Figure 5 compares the *let-7d* expression calculated by the ΔC_t method with the quantified *HMGA2* expression in the same samples. The correlation between the two sample sets was very poor at R^2=0.045 and P=0.86, indicating that there is no relationship between *HMGA2* gene expression in AAD tissue and *let-7d* (Fig 5).

Discussion

To the best of our knowledge, this is the first report of an alteration of *high mobility group AT-hook 2* (HMGA2) levels in aortic dissection. In this study we present evidence that the *HMGA2* gene is significantly upregulated in patients with type A aortic dissection when compared to other aortic diseases such as thoracic aortic aneurysm. The difference of *HMGA2* expression between the AAD samples and all the other investigated tissues "without dissection" was highly significant in the Mann-Whitney U-test at $P=1.4*10^{-7}$.

We investigated in this study a whole of 51 patients (19 AAD, 26 TAA, two Marfan syndrome and four aortic valve) and acknowledge that the 19 studied cases of acute aortic dissection type A were composed of 9 male and 10 female individuals, which contradicts data in the literature that reports acute aortic dissection as being 2.8 times more common in males than in females (Grundmann et al., 2006).

We found the female AAD patients to be of significantly higher age than their male counterparts (67.8±11.0 vs 50.4±18.0 years, *P*=0.03). This might be attributed to the functions of estrogens, which protect females from cardiovascular disease. Depletion of estrogen after the menopause leads to an increase of cardiovascular diseases in females of advanced age and might explain the fact that all female dissection cases in this study were 51 years old and above (Tan and Pu, 2004). Female patients featured also a significantly higher *HMGA2* expression (296.0±337.0 vs. 79.0±110.0 fold, *P*=0.02), which might relate to their higher age.

In the immunohistochemical analysis we determined the expression site of HMGA2 in AAD tissue. This protein is mainly found in cells surrounding the *vasa vasorum*. The *vasa vasorum* penetrates the *tunica media and tunica adventitia* for nutrition of the aortic layers. Immunohistochemistry of the endothelial marker CD34 revealed that the *HMGA2*-positive cells were endothelial, while the EMT marker protein SNAI1 was also found mainly in these cells. Additionally, Vimentin, member of the intermediate filament family, whose expression is upregulated during the transition of endothelial cells to mesenchymal cells, was detected in the *vasa vasorum* endothelium of AAD patients by immunohistochemistry and fluorescence double labeling. Expression of N-Cadherin, a secondary EMT marker, could not be found in fluorescence double labeling. N-Cadherin should be detectable at least in vascular smooth muscle cells as reported by various studies (Moiseeva, 2001). However the N-Cadherin antibody was proven to be functional in control tissue, so the meaning of this observation remains unclear. The presence of the EMT markers SNAI1 and Vimentin in endothelial cells indicates a possible endothelial-to-mesenchymal transition (EndMT), a form of EMT of the *vasa vasorum* endothelial cells. The absence of MKI-67 in the endothelium of the *vasa vasorum* suggests that no increased cell proliferation was detectable. This means that *HMGA2*, SNAI1 and Vimentin are expressed independently of cell proliferation. Aortic dissections arise from an entry tear in the aortic *tunica intima* or by intramural hematoma from rhexis of aortic *vasa vasorum* (Nienaber and Sievers, 2002; Tsai et al., 2005). EndMT in the *vasa vasorum* could lead to a loss of endothelial cells and a weakening of the endothelium, which would

render the *vasa vasorum* more prone to rupture.

The *Let-7d* microRNA was quantified in AAD patients and compared to *HMGA2* mRNA expression. Quantification at the mRNA level seems reasonable since human microRNA, unlike the *C. elegans* or *Drosophila counterparts*, tends to degrade its target mRNA completely (Smalheiser and Torvik, 2004). Moreover, a negative correlation between *let-7a* miRNA and *HMGA2* has already been found in gastric cancer cell lines (Motoyama et al., 2008). In our case no significant correlation was found between *HMGA2 and let-7d,* which suggests that *HMGA2* is expressed in a *let-7d*-independent manner in these patients. A possible explanation for this is the regulation of *HMGA2* by another *let-7* variant. It is also reasonable to assume that *let-7d*-independent *HMGA2* expression occurs only in patients with AAD, and that dysfunction of the *let-7* regulatory effect might be the reason for the overexpression of *HMGA2* in AAD. Alternatively, in these cases upregulation may occur at the transcriptional level.

In Marfan syndrome *Fibrillin-1*, a gene coding for a microfibrillar protein of the extracellular matrix, was found to be mutated (Dietz et al., 1991; Dietz and Pyeritz, 1995). Microfibrils composed of *Fibrillin-1* associate with the latent TGFB binding protein (LTBP), which controls the availability of TGFB in the extracellular matrix. Mutation of *Fibrillin-1* leads to dysregulation of the TGFB pathway and is therefore considered to be a critical event in the formation of AAD in Marfan syndrome (Neptune et al., 2003; Robinson et al., 2006). In a previous survey we found TGFB1 to be among the regulators of the differentially expressed genes in AAD (Mohamed et al., 2009). In this study we investigated a further 19 patients with AAD. They presented neither a clinical manifestation of Marfan syndrome nor mutation-related alterations in the *Fibrillin-1* gene. Of note, the TGFB1 protein was detected in massive amounts in almost all of the cells, including activated T cells and macrophages, and in all three layers of the aorta. However, we were not able to quantify the TGFB1 level due to its diffuse appearance. The signaling of TGFB has been implicated in pathways leading to enhanced extracellular matrix degradation and the production of various matrix metalloproteinase species (Kim et al., 2004). Since *HMGA2* acts downstream of TGFB in the EMT, the changing of the *vasa vasorum* by the transition of endothelial cells might be caused by TGFB dysregulation independent of a *Fibrillin-1* mutation.

Altogether these results suggest a possible remodeling of the *vasa vasorum* endothelium by the HMGA2-induced transition of endothelial cells to mesenchymal cells.

These mechanisms could lead to an impairment of the *vasa vasorum* and to the formation of intramural hematomas which are considered to be a predecessor of dissection (Nienaber and Sievers, 2002).

Alternatively the EndMT might be a repair mechanism as a consequence of tissue injury. Recent studies suggest a re-expression of *HMGA2* in pathophysiological adult tissues as a response to biochemical stress (Monzen et al., 2008).

Our results revealed for the first time the overexpression of the *HMGA2* gene in patients with AAD. This expression seems to be independent of regulatory miRNA *let-7d* and occurs mainly in the *vasa vasorum*. Since the EMT-causing transcription factor SNAI1 was expressed together with the EMT marker Vimentin in the endothelium of the *vasa vasorum,* the EndMT of these cells, induced by elevated *HMGA2* levels, seems possible. We assumed that upregulation of *HMGA2* seems to cause remodeling of the aortic wall by EndMT or it is a consequence of the aortic injury and repair activity following the acute events.

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Conflict of interest. None declared.

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