# GATA5 and Endothelial Nitric Oxide Synthase Expression in the Ascending Aorta Is Related to Aortic Size and Valve Morphology

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*Background.* **The pathogenesis of aortic dilatation in patients with congenital aortic valve anomalies is poorly understood. Recent studies suggest that alterations of gene expression may be related to ascending aortic aneurysm formation in these patients. Knockout of endothelial nitric oxide synthase (eNOS) and GATA5 is associated with bicuspid aortic valves in mice. To study the role of eNOS and GATA5 in human congenital aortic valve disease and aortic dilatation, we investigated their gene expression in aortic tissue from patients with unicuspid, bicuspid, and tricuspid aortic valves.**

*Methods.* **Samples from 84 patients (33 tricuspid, 32 bicuspid, and 19 unicuspid) were harvested intraoperatively from the ascending aorta. GATA5 and eNOS expression was determined by real-time polymerase chain reaction.**

*Results.* **GATA5 and eNOS expression in the aortic wall from patients with unicuspid aortic valves (GATA5: mean [M], 2.14; standard deviation [SD], 1.72; eNOS: M, 3.40; SD, 3.83) was significantly higher than in tricuspid aortic valves (GATA5: M, 1.12; SD, 0.80; eNOS: M, 1.00; SD, 0.74; each $p < 0.05$). Patients with bicuspid aortic valves (GATA5: M, 1.29, SD, 1.33; eNOS: M, 1.66; SD, 1.31) had a significantly higher eNOS expression than patients with tricuspid aortic valves ($p < 0.05$). The expression levels of eNOS and GATA5 correlated positively with each other and negatively with the ascending aortic diameter.**

*Conclusions.* **Our data suggest that GATA5, possibly through upregulation of eNOS, plays a role in the development of aortic dilatation in patients with unicuspid and bicuspid aortic valves. The differential gene expression in patients with unicuspid compared with bicuspid aortic valves suggests that the pathogenesis of both aortic valve anomalies may be different.**

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Bicuspid anatomy of the aortic valve (BAV) is the most frequent congenital cardiac anomaly [1, 2]. Patients with bicuspid aortic valves are more prone to develop ascending aortic dilatation irrespective of valve function [3, 4]. The high prevalence of aortic dilatation found in conjunction with BAV [5] has been related to the increased incidence of aortic dissection [4].

The pathomechanism of aneurysm formation in individuals with BAV is not well understood; a common genetic determination of bicuspid morphology and aneurysm formation has been assumed [6, 7]. That ascending aortic dilatation in patients with BAV is due to increased hemodynamic stress has also been proposed [8]. This, however, does not explain that aortic dilatation only occurs in a proportion of individuals with BAV; neither does it explain the two aneurysmal phenotypes: predominant dilatation of the tubular ascending aorta vs primary dilatation of the root [9]. Other studies indicate that structural deficiencies of the extracellular matrix and changes of protein expression within the aortic wall influence aortic dilatation in patients with BAV [10, 11].

Unicuspid aortic valves (UAV) are less frequent [12]. Compared with BAV, patients with UAV present with more severe valvular dysfunction and also aortic dilatation at an earlier age [4]. Whether these two anomalies are embryologically related or separate is unclear. Exact data on the true prevalence of aortic dilatation in UAV are missing; nonetheless, an increased probability of aortic dissection has also been described for this entity [4].

Although constitutional mutations in the NOTCH1 gene have been related to familial cases of BAV [13], similar relationships have not been identified for the nonfamilial majority of individuals. No defined genetic alterations have been associated with UAV so far.

Endothelial nitric oxide synthase (eNOS) has been suggested to be a determinant of BAV formation. Knockout of eNOS in mice led to a frequent development of BAV [7], even though aneurysm formation was not observed in this model. Double-knockout of eNOS and

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**Abbreviations and Acronyms**

ACE = angiotensin converting enzyme
ARB = angiotensin 2 receptor blocker
BAV = bicuspid aortic valve
BAVD = bicuspid aortic valve with aortic dilatation
BAVU = bicuspid aortic valve without aortic dilatation
cDNA = complementary deoxyribonucleic acid
EIF2B1 = eukaryotic transcription initiation factor 2B, subunit 1
ELF1 = ets-related transcription factor 1
eNOS = endothelial nitric oxide synthase
RQ = relative quantification values of the real-time PCR
TAV = tricuspid aortic valve
TAVD = tricuspid aortic valve with aortic dilatation
TAVU = tricuspid aortic valve without aortic dilatation
UAV = unicuspid aortic valve
UAVD = unicuspid aortic valve with aortic dilatation
UAVU = unicuspid aortic valve without aortic dilatation

apolipoprotein E caused formation of abdominal aortic aneurysms in mice [14].

eNOS has been shown to be regulated by GATA5, a transcription factor involved in cardiac development and formation of the outflow tract [15]. Interestingly, GATA5-knockout mice also show an increased prevalence of BAV, and eNOS expression is downregulated in their left ventricles and cardiac outflow tracts [15], without any known effect on aortic dilatation.

An interaction between GATA5 and eNOS still has to be proven in human congenital aortic valve disease. We previously showed that eNOS protein levels in the aortic wall of patients with BAV were lower compared with patients with tricuspid aortic valves (TAV) [6]. A negative correlation of eNOS protein levels and aortic diameter was found in BAV, but not TAV patients [6]. Nevertheless, it is uncertain whether the relationship between eNOS and aortic dilatation is a cause-and-effect type or whether downregulation of eNOS occurs as a consequence of the turbulence caused by BAV [16].

Nonsynonymous variations in the transcriptional activation domain of GATA5 have been reported in patients with BAV, indicating a possible role of GATA5 also in human BAV development [17]. The exact role of GATA5 in human valvulogenesis, however, still has to be defined. In addition, there is no information on the effect of GATA5 on aneurysm formation in patients with aortic valve malformations. If a relationship between GATA5, eNOS, and aortic dilatation could be identified, this would strengthen the hypothesis of eNOS being a determinant of the aortopathy seen in patients with BAV.

We therefore investigated the gene expression levels of GATA5 and eNOS in the ascending aortic wall tissue from patients with UAV, BAV, and TAV, with and without aortic dilatation.

## Material and Methods

This prospective study was conducted in accordance with the Declaration of Helsinki and was approved by the locally appointed Ethics Committee (Ethikkommission bei der Ärztekammer des Saarlandes, No. 205/10). All patients gave written informed consent.

### *Samples*

Specimens of ascending aortic tissue were obtained from 84 patients undergoing aortic valve operations with or without ascending aortic replacement. The tissue specimens were harvested at the convexity of the ascending aorta, 5 mm cranial of the sinotubular junction. Aortic dimensions were determined by transesophageal echocardiography using standard measurements [18]. The aortic valve morphology was assessed intraoperatively. The specimens were immediately snap frozen in liquid nitrogen and stored at –80°C.

The ascending aorta was considered dilated in case of a sinus or tubular ascending aortic diameter of 40 mm or larger. UAV was present in 19, BAV in 32, and TAV in 33 patients. The distribution of valve morphology and aortic dilatation of the patients is summarized in Table 1. Further clinical characteristics are reported in Table 2. Among the patients with BAV, 24 had a BAV with fusion of the right and left coronary cusp (15 dilated, 9 undilated), and 8 had a BAV with fusion of the right and noncoronary cusp (7 dilated, 1 undilated). According to the pattern of the dilated ascending aorta, the patients were divided into two groups: dilatation type 1 if the tubular aortic diameter exceeded the sinus diameter (32 patients) and dilatation type 2 if the sinus diameter exceeded the tubular aortic diameter (13 patients).

### *RNA Isolation and Complementary DNA synthesis*

RNA isolation was performed with the mirVana PARIS Kit (Ambion, Austin, TX). Frozen tissue samples were homogenized using an Ultra Turrax T8 homogenizer (Ika, Staufen, Germany) and an ultrasonic processor UP100H (Hielscher, Teltow, Germany). Further isolation was performed according to the manufacturer's recommendations. DNAse digestion and RNA cleanup were done with the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA quantity and quality were determined with an Infinite 200 NanoQuant (Tecan, Männedorf, Switzerland). RNA integrity was confirmed with an Agilent 2100 Bioanalyzer and the Agilent 6000 Nano Kit (Agilent Technologies, Santa Clara, CA).

Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations.

Quantitative real-time polymerase chain reaction was performed with TaqMan Gene Expression Assays (Applied Biosystems) for *GATA5* (Hs00388359_m1) and *NOS3* (Hs01574659_m1) on 96-well TaqMan Plates