2015

Discovering the link between bicuspid aortic valve and aortic aneurysms: genetic or hemodynamic?

https://hdl.handle.net/2144/16108

Boston University
DISCOVERING THE LINK BETWEEN BICUSPID AORTIC VALVE AND AORTIC ANEURYSMS: GENETIC OR HEMODYNAMIC?

by

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B.S., B.A., Providence College, 2012

Submitted in partial fulfillment of the requirements for the degree of Master of Science

2015
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ABSTRACT

Objectives

The association between bicuspid aortic valves and aortic aneurysms has been well documented. In order to better understand this association, this study sought to accomplish two goals. The first was to determine if there was any correlation between specific bicuspid aortic valve phenotypes and aortic aneurysms. The second goal was to determine if the association between bicuspid aortic valve disease and aortic aneurysms has a genetic or hemodynamic cause.

Methods

For the non-genetic portion of the study, we used echocardiogram and surgical records to classify the phenotypes of the aortic valve and the aorta of 434 patients. We then evaluated the correlation between valve morphotype and aortic aneurysm phenotype. For the genetic portion, we used a genome wide association study on 452 patients to find genes that could potentially be responsible for aortic aneurysms. These were then compared with genes suspected of causing bicuspid aortic valve to determine if there is a common genetic link between the two disorders.
Results

We observed a significant association between bicuspid aortic valve and aortic aneurysms; however we did not find any significant association between the different bicuspid aortic valve phenotypes and aortic aneurysm phenotypes. For the genome wide association study, we identified genes that could potentially be responsible for causing aortic aneurysms; however, none of the suspected markers were considered statistically significant. Also none of the identified genes matched to the genes suspected of causing bicuspid aortic valve.

Conclusion

While the results were not as expected, the study provided us with information to better understand the relationship between bicuspid aortic valves and aortic aneurysms. According to the results of the current study, patients with bicuspid aortic valve are more likely to develop an aortic aneurysm but specific phenotype has no effect on where the aneurysm occurs in the aorta. The increased frequency of aortic aneurysms in bicuspid valve patients is most probably due to a combination of altered hemodynamics and genetic effects. In order for this information to be useful in the clinical setting, the methods of this study should be repeated in a larger cohort to make sure the results are accurate.
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INTRODUCTION

The aortic valve and the ascending aorta

The aortic valve is a semilunar valve composed of three leaflets or cusps (left, right, and non coronary). Its function is to maintain unidirectional arterial flow by preventing regurgitation of blood from the aorta back into the left ventricle during diastole. During systole, as the pressure in the left ventricle exceeds the pressure in the aorta, the aortic valve opens and allows the left ventricle to eject blood into the aorta; resulting in organ perfusion. The tri-leaflet structure of the valve is an optimal engineering configuration that allows high-velocity flow with minimal pressure loss across the valve during systole, while having perfect occlusion during diastole. Leonardo da Vinci first recognized this fundamental engineering principle in the early 1500s.

Figure 1. The aortic valve. The orientation of the aortic valve during systole (open) and diastole (closed) (Figure taken from AllinaHealth).
The aorta is the main artery of the systemic circulation. Histologically the aorta can be broken up into three layers. The innermost layer is termed the intima and is made up of the endothelium and it underlying elastic lamellae. Its function is to create a non-thrombogenic surface to minimize activation of the coagulation and inflammatory pathways. The thickest and middle layer of the aorta is termed the media and is made up of both smooth muscle cells positioned perpendicular to the lumen and an extensive amount of elastic tissue. The elastic proteins of the aorta, composed of proteins such as collagen, elastin, fibrillin, etc., are secreted and maintained by the smooth muscle cells of the tunica media (Stegemann et al., 2004). The principal function of the tunica media is to provide
structural integrity to the aorta allowing the aorta to stretch during systolic pressure increases. The outermost layer is termed the tunica adventitia and is made up of collagen and a supporting elastic layers.

Anatomically the aorta is divided into several sections based on its anatomy: the ascending aorta, the aortic arch, the descending aorta, and the abdominal aorta. The ascending aorta begins at the aortic valve, forming an aortic root where the coronary arteries take their origin, with a tulip-like expansion that appears to have an optimal design for blood flow through the aortic valve. The opening from the left ventricle into the aorta (at the level of the aortic valve) is termed the annulus, the widest part of the aortic root is termed the sinus of Valsalva, and the transition from the aortic root to the tubular proximal ascending aorta is termed the sinotubular junction.

**Bicuspid aortic valve**

The normally tri-leaflet valve aortic valve is occasionally formed with two leaflets resulting in a bicuspid valve. The bicuspid aortic valve (BAV) is the most common congenital cardiac anomaly, with an incidence of 0.9% to 1.5% in the general population and is more frequent in males and Caucasians (Della Corte et al., 2014). Clinically a patient with BAV can be diagnosed with three different phenotypes depending on which two leaflets are fused, each with its own distinct morphological appearance and physiological effects. These groups are defined
by the fusion of two leaflets of the aortic valve and they are Left-Right fusion (L-R), Right-Non Coronary fusion (R-NC), and Non Coronary-Left fusion (NC-L).

![Diagram of bicuspid valves](image)

**Figure 3. Different phenotypes of bicuspid valves.** A, L-R BAV. B, R-NC BAV. C, NC-L BAV. D, L-R BAV with two equal sized leaflets. E, L-R true BAV. F, L-R BAV with incomplete raphe (Figure taken from Mechelena et al., 2014).

Pure or true BAV presents with two, usually completely symmetrical cusps (Figure 3E). The fusion can be either vertical or horizontal and it can fall into any of the three phenotypes listed above. However this type accounts for only 7% of BAV patients (Sievers et al., 2007). The remaining patients with BAV present with a non-symmetric valve (Figures 3A, B, C). During these patients’ embryonic development, two leaflets fuse creating a single leaflet that is usually much greater in size than the remaining, non-fused leaflet with a fibrous ridge (a raphe)
marking the fusion of the two leaflets. L-R fusion is the most common type and is present in 72% of patients with BAV. R-NC fusion is seen in about 25% of patients and NC-L fusion is seen in about 3% of patients. In about 1% of the BAV population, a combination of both L-R fusion and R-NC fusion results in a valve that appears to be a single leaflet, called a unicuspid valve (Sievers et al., 2007). These categories are clinically important because of the differing physiological and hemodynamic effects of each BAV type.

While L-R, R-NC, and NC-L are the major classification groups used for BAV patients, additional anatomical and physiological characteristics are used to describe the valve structure and its function. These include leaflet symmetry, raphe completeness, valve incompetence, stenosis, and/or calcification. Leaflet symmetry is based on the size of the two cusps and in the majority of BAV patients they are asymmetric. Raphe completeness describes the appearance of a raphe and can be either complete or incomplete. Incompetence of the aortic valve occurs when the leaflets fail to coapt (close completely) during diastole resulting in regurgitant flow back through the valve in diastole. Aortic stenosis occurs when the aortic valve is markedly calcified, thickened and fails to open fully during systole resulting in turbulent flow and pressure loss.

**Ascending aortic aneurysms**

An aortic aneurysm is an abnormal widening, usually by more than 50% of the normal diameter, of a portion of the aorta due to degeneration of the media of
the aortic wall (Goldinger et al., 2014). Aortic aneurysms can occur anywhere on
the entire length of the aorta however 60% of aortic aneurysms involve the aortic
root or the proximal ascending aorta (Isselbacher et al., 2005). On a cellular
level, medial degeneration results from the injury and loss of smooth muscle cells
and degradation of the elastic fibers (Isselbacher et al., 2005). This leads to a
decrease in the vessel wall integrity and decreases the aorta’s ability to manage
pulsatile pressure changes. As distensibility decreases, the aortic wall will
eventually reach its tensile limit, which can lead to aortic dissection or rupture.

Aortic dissection is the separation of the planes of the aorta, initially
between the intima and medial portions that leads to intramural hemorrhage in
the aorta and can eventually lead to aortic rupture. Aortic dissection is a life-
threatening condition with a grim prognosis and has been described as “the most
devastating complication of thoracic aortic disease” (Goldinger et al., 2014).
Once the aorta has ruptured, less than half of patients survive. Because aortic
aneurysm and dissection are so lethal, it is important to identify patients who
suffer from aortic aneurysm and to determine ways to predict and prevent
dissection and rupture.

Measurement of aortic dimensions is normally performed using
echocardiography or computed tomography imaging; however, determining
whether or not a patient has developed an aortic aneurysm is more difficult as
there is usually no longitudinal imaging for a single patient. Thus definitions of
aortic aneurysm rely on population means, adjusted for age and patient size.
Usually clinicians use a normalizing formula that requires a patient’s BSA to determine if the aorta is aneurysmal and some even use a standard number of 35 millimeters to determine whether or not there is an aneurysm present (Roman et al., 1989). However it may be more useful to develop a better method of normalizing healthy aortic diameter in order to determine if the patient is at risk of an aortic aneurysm (Davies et al., 2006).

In an attempt to classify patients based on their ascending aortic aneurysm phenotype, three classification methods have been proposed. The method most commonly used is the one proposed by Schaefer et al. (2007). This method defines the shape of the aorta by considering the dimensions of the ascending aorta sections regardless of dilatation. This method uses three classes: the N shape (ascending aorta < sinuses > STJ), the A shape (ascending aorta > sinuses > STJ), and the E shape (sinuses < STJ, regardless of ascending aorta size). A second classification scheme proposed by Park et al. (2011) depends of the appearance in dilatation at the root, the proximal ascending aorta, or both. This method also uses three classes and they are: type 1 (dilatation of the ascending aorta only), type 2 (dilatation at both the proximal ascending aorta and the root), and type 3 (dilatation of the root only). The most recent classification method was proposed by Della Corte et al. (2013). This method uses only two classification types and they are: root phenotype or ascending phenotype (depending on which portion is predominantly involved).
Figure 4. Different phenotypes of ascending aortic aneurysms. Picture representation of the different classification systems for ascending aortic aneurysm phenotypes (Figure taken from Della Corte et al., 2014).
A higher prevalence of ascending aortic aneurysm in patients with a bicuspid aortic valve

There is an association between bicuspid aortic valves and aortic aneurysm and aortic dissection (Schaefer et al., 2007). As a group, BAV patients have larger average measurements in each section of the aorta (annulus, sinus and proximal ascending aorta) when compared to patients with tricuspid aortic valves (Nkomo et al., 2002). In addition, it has been noted that specific BAV phenotypes have a tendency to lead to a specific aortic aneurysm phenotype (Della Corte et al., 2014). Patients with the most common form of BAV, R-L fusion, have a higher incidence of aortic root aneurysms (Schaefer et al., 2007). The R-N BAV phenotype has an association with proximal ascending aortic aneurysms. In a study of 622 BAV patients, R-L phenotype was associated with dilatation at the sinus in 41% of patients versus 22% of patients with R-N phenotype. Fifty six percent of patients with R-L phenotype had dilatation at the ascending aorta where as 68% of patients with R-N phenotype had this type of dilatation (Della Corte et al., 2014). These studies show the importance of consistent classification and terminology for both researchers and clinicians who study BAV. Clearly not all BAV patients are the same and each requires different treatment and management based on phenotype and other factors, including age, sex, body surface area, etc. Unfortunately, a recent survey among cardiac surgeons has shown that not all clinicians are aware of the findings of studies like the one above (Della Corte et al., 2014). According to the aforementioned
survey, “only 15% of the respondents were aware that the pattern of valve leaflet fusion is associated with a unique pattern of aortic dilatation”.

Histologically, it has been shown that patients with BAV have accelerated deterioration of the aortic wall (Schmid et al., 2002). Patients with BAV showed a greater amount of infiltrating leukocytes and depleted numbers of smooth muscle cells due to apoptosis. Tissue samples from aneurysmal aortas in BAV patients showed expression of apoptotic mediators perforin and Fas/FasL, both of which were not present in normal aortic tissue. Researchers have also found increased destruction of the elastic laminae in the aortic wall of BAV patients. All of these findings have direct correlation with increased incidence of aortic aneurysm (Schmid et al., 2002). Mediators of apoptosis secreted by infiltrating leukocytes caused increased apoptosis of the smooth muscle cells, which are normally responsible for maintenance of the connective tissue in the media of the wall of the aorta. In turn, this causes an alteration of the extra cellular matrix leading to weakening of the elastic and tensile properties of the aorta potentially resulting in aortic aneurysm and dissection (Forte et al., 2013).

While there is little debate on the correlation between BAV and aortic aneurysm, there is debate among clinicians and researchers regarding the etiology of aortic aneurysms; whether there may be hemodynamic causes of aortic aneurysm that act independently of the genetic causes of aortic aneurysm. BAV prevalence is “nearly 10-fold higher in primary relatives of patients with BAV than in the general population” (Prakash et al., 2014). Although the genetic
causes of BAV have not been clearly identified, by studying familial pedigrees it is believed that BAV is inherited in an autosomal dominant fashion. Genes such as \textit{NOTCH1}, \textit{ACTA2} and a few others have been determined to play a part in causing BAV; however, it is not entirely clear how much influence these genes have and whether or not they work in conjunction with other, currently unidentified, genes (Prakash et al., 2014). Researchers are continuously conducting studies with large cohorts of BAV patients to uncover more information about the genetics involved in this disorder.

As investigators continue to look for genes that cause BAV, it has been speculated by some that these same genes are also responsible for some of the complications associated with BAV, specifically aortic aneurysm (Prakash et al., 2014). Although specific genes such as \textit{FBN1} have been linked to aortic aneurysms, these are different from the genes that are believed to be responsible for BAV. However, there is evidence that suggests that the genes that cause BAV could also be directly linked to aortic aneurysms as well. Two arguments in favor of a genetic correlation between BAV and aortic aneurysms are the number of BAV patients who develop aneurysms and the similar embryological origin of both the aortic valve and the ascending aorta. Researchers who believe there is a genetic link cite the fact that “because BAV and TAAD frequently occur together, the genetic architecture of BAV may also consist of many different genetic variants that interact in an additive manner to increase risk for BAV and its complications” (Prakash et al., 2014). Because the
association between BAV and TAAD is so strong, it is a reasonable hypothesis that they have a common etiology. Further supporting the hypothesis is the common embryological origin of the aortic valve and ascending aorta. Both the aortic valve and ascending aorta develop from neural crest cells early in fetal development (Cotrufo et al., 2005). It is reasonable to believe that a genetic defect specifically altering the function and development of neural crest cells could result in defects of both the aortic valve and the media of the ascending aorta.

While there is a possibility of a genetic link between BAV and aortic aneurysms, some researchers believe that the link is related to the fact that bicuspid aortic valves have altered biomechanics (Conti et al., 2010). BAV patients are at greater risk of aortic leaflet thickening, calcification and stenosis resulting in abnormal flow patterns in the aortic root and ascending aorta. Fusion of two leaflets causes restrictions in the opening of the valve and thus the valve opening becomes more elliptical, and off center (Conti et al., 2010). This restricted cusp motion leads to blood flow deflection not seen in patients with normal aortic valves (Della Corte et al., 2012). According to Bissell et al (2013), as the valve opening becomes more narrowed, the blood flow into the aorta becomes more helical and less cohesive. As a result, the blood stream hits the aortic wall at an abnormal angle causing increased local wall shear stress. They hypothesize that aortic dilation is a compensatory mechanism to combat the increased stress caused by the abnormal blood flow. Increased wall shear stress
has been shown to cause increased levels of matrix metalloproteinase and other cellular enzymes that lead to extra cellular matrix remodeling that will eventually lead to aortic aneurysms (Stegemann et al., 2004). Increased sheer stress has also been shown to have a negative effect on vascular smooth muscle cells, causing a decrease in proliferation and an increase in apoptosis, which can lead to destruction of the aortic wall (stated above) (Stegemann et al., 2004).

**Surgical repair of aortic valves and aortic aneurysms**

Dysfunctional aortic valves can be surgically replaced. The two main reasons for valve replacement procedures are stenosis, the narrowing of the valve opening causing obstruction of blood flow due to thickened valve leaflets, or regurgitation, the back flow of blood into the left ventricle because of valve prolapse. Stenosis is usually the result of decades of aortic stress and calcium deposition on the valves from normal blood flow causing loss of mobility of the leaflet. Regurgitation is typically caused by leaflet failure due to the degeneration of the connective tissue or by changing pressure in the aorta due to dilatation. During an aortic valve replacement procedure, the valve is removed and replaced with a prosthetic valve. The replacement valve can be either biological in origin (usually porcine or bovine) or mechanical. Prosthetic valves typically last for 10-20 years and this type of surgery usually has a very low mortality rate (Opotowsky et al., 2013). In addition, the life expectancy of a patient suffering from severe stenosis and/or regurgitation is much longer if the valve is replaced.
Patients with congenital BAV are at higher risk for both stenosis and regurgitation and patients with this condition usually require a valve replacement surgery at some point in their lifetime ("Heart Surgery - Aortic Valve Surgery," n.d.).

Similarly, aortic aneurysms require reparative surgery to prevent more serious aortic events such as dissection and rupture, which can be fatal. Once an aneurysm has been deemed to be too large (usually when the size reaches 5 cm or increases by 0.5 cm in a year), surgery is required to repair the aneurysm. During this procedure, the aneurysmal portion of the aorta is excised and replaced with a synthetic graft ("Aortic Aneurysm Surgery," n.d.). Depending on where the aneurysm occurs on the aorta, this procedure can be done while sparing the aortic valve or may require an aortic valve replacement at the same time. Like the aortic valve replacement surgery, this procedure usually has very low operative risks (Opotowsky et al., 2013).

This is where the importance of BAV classification becomes apparent. Most BAV patients will need a valve replacement due to severe stenosis, calcification or some other pathology that renders the valve physiologically inadequate. Additionally, 30% of BAV patients will develop an aortic aneurysm and require surgical replacement of a piece of their aorta (Prakash et al., 2014). If the research presented above is correct, and it is confirmed that patients with specific BAV phenotypes have a tendency to develop a specific aortic aneurysm phenotype, this could lead to major changes in the methods used to treat and manage BAV and its related health effects. Most specifically, clinicians can tailor
a BAV patient’s treatment and surgical procedures based on their specific phenotype of BAV. For example, if a patient presents with a R-L phenotype, he/she is more likely to develop an aneurysm at the level of the Sinus of Valsalva, which tends to occur at a younger age (Della Corte et al., 2014). This patient will require a stricter clinical surveillance and different surgical arrangements (surgery at a younger age, replacement valve type, etc.) when compared to a patient with R-N phenotype who is less likely to develop a root phenotype aneurysm.

Furthermore, if it is confirmed that genes suspected of causing BAV truly do so, this may also lead to a better, more refined approach to monitoring, managing, and treating BAV and its associated health effects. If managed from a younger age, it may be possible to prevent or slow the progression of these adverse effects such as stenosis, calcification, or valve failure. Also, with earlier treatment and/or preemptive valve replacement surgery, it may be possible to slow down or even prevent BAV related aortic aneurysms.

**Current study**

The current study aims to provide insights that will one day be used to advance the clinical management/treatment of BAV. We hope to achieve this aim by completing two objectives. First, confirm the association between BAV phenotype and aortic aneurysm phenotype and second, determine whether the association between BAV and aortic aneurysms has a genetic link or a
hemodynamic cause.

The purpose of the first objective is to confirm the results of a previously mentioned study in hopes to support the installment and use of a universal classification system. The benefits of using a universal classification system have been discussed above and if the results of the current study confirm the association between different BAV phenotypes and ascending aneurysm phenotypes, this would support the use of such a system. In order to do so, we will analyze echocardiogram images of the aortic valve and ascending aorta to determine BAV and aortic aneurysm phenotyping. Then we will use statistical analysis to determine whether or not there is a direct correlation between BAV phenotype and aortic aneurysm phenotype.

The purpose of the second objective is to help provide an answer to the debated question about the correlation between BAV and aortic aneurysms. By screening our participants for the genes that could potentially cause aortic aneurysms, we hope to identify a genetic cause and to determine if these same genes are somehow involved in BAV. We hope that the results of the current study will provide enough data to advance the treatment of BAV. Regardless of the outcomes, our main objective is to provide information that will one day lead to better clinical management of BAV in order to improve the lives and health of those who suffer from this common congenital disorder.
MATERIALS AND METHODS

Patients

After local Institutional Review Board approval, patients were identified from a database of >10,000 potential bicuspid aortic valve patients collected between 2010 and 2015 through a variety of different sources (the majority of which came from a research patient data registry at Brigham and Women’s Hospital). Each patient was contacted and, of these 10,000 patients, 1609 patients consented to providing a DNA sample. For the present study, we selected patients who have had aortic valve replacement surgery or another type of cardiac surgery at a Partners-affiliated hospital (principally Brigham and Women’s Hospital and Massachusetts General Hospital) because we had greatest access to medical records for these patients and thus the necessary data was much more accessible for these patients. The exclusion criteria were: systemic disorders (Marfan syndrome, Turner syndrome, etc.), age greater than 80 at the time of surgery, and/or previous cardiac surgery. In total, 730 patients were eligible for inclusion in the study.

For the non-genetic portion of the study, we removed any patient that did not have a clear description of the aortic valve phenotype and aortic aneurysm phenotype. Patients with NC-L fusion were not considered in the study because of the rarity of this phenotype. The total number of remaining patients for the non-genetic portion was 434. For the genetic portion of the study, 452 patients with
clear records of aortic aneurysm phenotype were available. There was an overlap of 227 patients in the two arms of the study.

Valve classification

Valve phenotype was classified as L-R (fusion between left and right leaflets), R-NC (fusion between right and non-coronary leaflets), or not bicuspid (tricuspid valves with no leaflet fusion). The bicuspid aortic valve was confirmed by visualization of the aortic valve when seen in an axial view of a transesophageal or transthoracic echocardiogram in 311 patients. For the remaining patients, no TEE or TTE images were available so the phenotype of the aortic valve was arbitrated from echocardiogram or surgical report.

Aortic aneurysm classification

The aortic aneurysm phenotype was measured from echocardiogram or CT dimensions and classified as root (dilatation at the sinus of Valsalva), ascending (dilatation at the proximal ascending aorta), or no aneurysm. For the 311 patients with echocardiogram images, the aorta was measured in the coronal view of a TEE or TTE. The sinus and proximal ascending aorta was measured from inner edge to inner edge during diastole. The aorta was determined to be aneurysmal if the ratio of the patient’s measured aortic diameter to the patient’s normal aortic diameter exceeded 1.15. In order to determine each patient’s normal aortic diameter we used the Roman formulas
and to determine BSA (needed for the Roman formulas) we used the Dubois formula (Roman et al., 1989). For the remaining patients, no TEE or TEE images were available so aortic aneurysm was arbitraged from echocardiogram or surgical report.

**Genome wide association study**

All 452 patients used for the genetics portion of this study were genotyped using the Illumina HumanOmni2.5 BeadChip. Three dbGap cohorts (Framingham Heart Study, Genetic Epidemiology of Refractive Error in the KORA, The Genetic Architecture of Smoking and Smoke Cessation, Joint Addiction, Aging, and Mental Health) were used as control (tricuspid) patients and all were genotyped with either Human Omni2.5 or HumanOmni5.0 arrays. The quality control steps of the genotype data are listed in the table below (Table 1).

**Statistical analysis**

For the non-genetic portion of this study, valve phenotype frequencies were compared by chi-square test, likelihood ratio chi-square test, and Mantel-Haenzel chi-square test for both aneurysm phenotypes. Logistic regression analyses were performed to check the significance level of both BAV phenotypes in predicting an aneurysm of either phenotype compared to TAV and to calculate the odds ratios. P values were adjusted for age. A difference of least squares means test was used to determine if there was any significant distinction
between the two BAV phenotypes in predicting an aneurysm of either phenotype.

For the genetic portion of this study, logistic regression analysis was used to find association with aortic aneurysm while accounting for age and gender as covariates.

Table 1. Quality control of genotype data

<table>
<thead>
<tr>
<th>Quality Control steps</th>
<th>Filters</th>
<th>BAV case cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw genotype data</td>
<td>Read (Bim, Bed, Fam) for 480 samples</td>
<td>2,379,855 markers</td>
</tr>
<tr>
<td>Step1</td>
<td>Exclude markers with no-founders</td>
<td>2,802 markers</td>
</tr>
<tr>
<td>Step2</td>
<td>Exclude markers with no-position</td>
<td>7,238 markers</td>
</tr>
<tr>
<td>Step3</td>
<td>Exclude monomorphic markers</td>
<td>33 markers</td>
</tr>
<tr>
<td>Step4</td>
<td>Exclude pseudo-autosomal region of X</td>
<td>418 markers</td>
</tr>
<tr>
<td>Step5</td>
<td>Exclude mitochondrial chromosome</td>
<td>256 markers</td>
</tr>
<tr>
<td>Step6</td>
<td>Exclude X and Y chromosomes</td>
<td>57,769 markers</td>
</tr>
<tr>
<td>Step7</td>
<td>Exclude markers if missing rate &gt; 10%</td>
<td>8,076 markers</td>
</tr>
<tr>
<td>Step8</td>
<td>Exclude markers with MAF&lt;5%</td>
<td>1,053,599 markers</td>
</tr>
<tr>
<td>Step9</td>
<td>Markers failed HWE test (P&lt;0.0001)</td>
<td>3,942 markers</td>
</tr>
<tr>
<td>Step10- for samples</td>
<td>Removed samples with low genotype rate (&lt;95%)</td>
<td>16 samples</td>
</tr>
<tr>
<td>Step11- for samples</td>
<td>Population stratification – detected outliers</td>
<td>8 samples</td>
</tr>
<tr>
<td>Step12- for samples</td>
<td>Remove samples with no-BAV phenotype</td>
<td>4 samples</td>
</tr>
</tbody>
</table>

Total SNPs and samples which remained after QC = 1,245,722 SNPs and 452 samples
RESULTS

Correlation between aortic valve phenotype and aortic aneurysm phenotype

Of the 434 patients used in this portion of the study, 262 (60.3%) had a L-R fusion pattern, 62 (14.2%) had a R-NC fusion pattern, and 110 (25.3%) had a tricuspid aortic valve (Table 2). For L-R fusion phenotype, 37 of the 262 patients (14%) presented with a root phenotype aneurysm. For R-NC fusion phenotype, 5 of the 62 patients (8%) presented with a root phenotype aneurysm. For the TAV patients, 10 out of 110 (9%) presented with a root phenotype. In total, 52 of the 434 total cases (12%) presented with a root aneurysm phenotype (Table 2).

Only 404 patients were used in determining the frequencies of proximal ascending aortic aneurysms because 30 patients did not have the necessary data to accurately determine if an aneurysm was present or not. For L-R phenotype, 130 out of 258 patients (50%) were identified as having an aneurysm in the proximal ascending aorta. For R-NC phenotype, 37 of the 62 patients (60%) and 22 of the 84 TAV patients (26%) presented with this type of aneurysm (Table 3). In total, 189 of the 404 cases had an ascending aneurysm phenotype.
Table 2. Frequency of Aortic Root Aneurysm

<table>
<thead>
<tr>
<th>Valve</th>
<th>Aortic root aneurysm present</th>
<th>Not present</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-R</td>
<td>37 (14%)</td>
<td>225 (86%)</td>
<td>262</td>
</tr>
<tr>
<td>R-NC</td>
<td>5 (8%)</td>
<td>57 (92%)</td>
<td>62</td>
</tr>
<tr>
<td>TAV</td>
<td>10 (9%)</td>
<td>100 (91%)</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>52 (12%)</td>
<td>382 (88%)</td>
<td>434</td>
</tr>
</tbody>
</table>

Table 3. Frequency of Ascending Aortic Aneurysm

<table>
<thead>
<tr>
<th>Valve</th>
<th>Ascending aortic aneurysm present</th>
<th>Not present</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-R</td>
<td>130 (50%)</td>
<td>128 (50%)</td>
<td>258</td>
</tr>
<tr>
<td>R-NC</td>
<td>37 (60%)</td>
<td>25 (40%)</td>
<td>62</td>
</tr>
<tr>
<td>TAV</td>
<td>22 (26%)</td>
<td>62 (74%)</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>189 (47%)</td>
<td>215 (53%)</td>
<td>404</td>
</tr>
</tbody>
</table>

We observed a significant association between valve phenotype and proximal ascending aortic aneurysm phenotype (p<0.0001) but no association between valve phenotype and aortic root aneurysm phenotype (p=0.23). Using TAV phenotype as a reference, L-R and R-NC valves are significantly associated with the presence of a proximal ascending aneurysm. Patients with L-R valve
phenotype were three times more likely to have a proximal ascending aortic aneurysm and patients with a R-NC were four times more likely to have this type of aneurysm. These differences persisted despite adjustment for age and gender as covariates. By contrast, there was no observed association between valve phenotype and aortic root aneurysm.

**Identification of loci associated with root and proximal ascending aneurysms**

The GWAS was conducted on 262 cases for ascending aneurysm and 101 cases for root aneurysm. 29 SNPs on chromosomes 8, 10, 11, 13, 14, 15, and 16 were found to have an association with proximal ascending aortic aneurysm (Table 5) and 81 SNPs on chromosomes 2, 8, and 11 were found to have an association with aortic root aneurysm (Table 4). However none of these SNPs showed significance at the GWAS level (p < 5*10^{-8}).

Table 4. Top SNPs for root aneurysms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>OR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs13013272</td>
<td>2</td>
<td>2.3</td>
<td>1*10^{-4}</td>
</tr>
<tr>
<td>rs10108063</td>
<td>8</td>
<td>2.5</td>
<td>1*10^{-5}</td>
</tr>
<tr>
<td>rs4937879</td>
<td>11</td>
<td>2.1</td>
<td>2.4*10^{-5}</td>
</tr>
</tbody>
</table>
### Table 5. Top SNPs for proximal ascending aneurysms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>OR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12716962</td>
<td>16</td>
<td>2.1</td>
<td>1.2*10^-5</td>
</tr>
<tr>
<td>rs4505047</td>
<td>11</td>
<td>2.0</td>
<td>2.8*10^-5</td>
</tr>
<tr>
<td>rs4905932</td>
<td>14</td>
<td>0.54</td>
<td>9.7*10^-5</td>
</tr>
<tr>
<td>rs2812743</td>
<td>13</td>
<td>0.47</td>
<td>2.6*10^-5</td>
</tr>
<tr>
<td>rs10887104</td>
<td>10</td>
<td>0.33</td>
<td>2.5*10^-5</td>
</tr>
<tr>
<td>rs959692</td>
<td>8</td>
<td>1.8</td>
<td>1.1*10^-4</td>
</tr>
</tbody>
</table>

### Table 6. Top SNPs common in both BAV and aortic aneurysms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Aneurysm Phenotype</th>
<th>OR (for aneurysm)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs789663</td>
<td>Ascending</td>
<td>0.49</td>
<td>6.4*10^-5</td>
</tr>
<tr>
<td>rs2868750</td>
<td>Ascending</td>
<td>0.38</td>
<td>8.3*10^-5</td>
</tr>
<tr>
<td>rs7952073</td>
<td>Root</td>
<td>2.1</td>
<td>6.5*10^-5</td>
</tr>
<tr>
<td>rs12274692</td>
<td>Root</td>
<td>2.1</td>
<td>7.4*10^-5</td>
</tr>
</tbody>
</table>
DISCUSSION

Association between BAV phenotype and aortic aneurysm phenotype

Association between bicuspid aortic valve and aortic aneurysms has been previously identified (Schaefer et al., 2007) and similarly, association between different BAV phenotypes and different aortic aneurysm phenotypes has been documented in the literature, with L-R valve morphology being associated with root aneurysm phenotype and R-NC valve morphology being associated with proximal ascending aneurysm phenotype has (Della Corte et al., 2014). We sought to replicate these latter findings but were unable to do so.

We did confirm an association between BAV and aortic aneurysm in our cohort with an approximately two-fold increased risk in this surgical population. This was observed for both BAV phenotypes: 142 (54%) L-R patients had an aneurysm and 38 (61%) of R-NC patients did as well.

While there is a clear indication that BAV patients are at greater risk to develop an aortic aneurysm, once the cohort was broken down by valve phenotype and each aneurysm phenotype was considered separately, the association was not as clear. When considering the risk of developing a proximal ascending aneurysm, BAV patients were at a greater risk than TAV patients. Importantly, when considering L-R and R-NC patients separately, both BAV morphotypes showed a significantly increased risk for developing a proximal ascending aneurysm when compared to TAV patients. The data from the current
study suggests that L-R BAV patients have a 3-fold increased risk and R-NC patients have a 4-fold increased risk when comparing to TAV patients. However, when testing our hypothesis that the valve phenotype had impact upon the aortic phenotype, we did not observe an association, which was unexpected. As stated above, it has been shown that the R-NC valve phenotype has a greater association with proximal ascending aortic aneurysms. While our statistical analysis did not find a significant difference between L-R and R-NC for this type of aneurysm, the frequencies in our cohort and relative risk showed that R-NC patients have an increased incidence of proximal ascending aneurysms when compared to L-R. Sixty percent of R-NC patients presented with a proximal ascending aneurysm with an OR of 4.4 whereas L-R patients had a 50% frequency and an OR of 3.0. Based on these results, there appears to be a difference between the two valve phenotypes in predicting this type of aneurysm.

Limitations to our examination of this hypothesis may have included inadequate sample size as mentioned above or confounding by the mode of presentation in the study population. The examined cohort was not a population wide sample and the majority of patients were enrolled because they sought care for aortic valve disease or aortic aneurysmal disease. Nevertheless, the important finding is that no matter which phenotype, BAV patients are at a much greater risk of developing a proximal ascending aneurysm when compared to the normal population. Whether or not there is a difference between L-R and R-NC, both phenotypes presented with a frequency of incidence of greater than 50%.
Despite the fact that R-NC had a higher frequency, the frequency in L-R was high as well. Thus, the likelihood of either patient set benefitting from a different course of clinical care is highly unlikely. No matter what BAV phenotype, patients will still have to be monitored closely for proximal ascending aneurysms.

When considering root aneurysms, the current study shows that there is no significant difference between any of the three valve phenotypes studied. Again, based on the outcomes of previous studies, these results were unexpected. Others have shown that patients with L-R BAV phenotype are at a greater risk of developing a root phenotype aneurysm; the failure of the association to reach statistical significance in our study suggests that this is not the case. But as mentioned above, this may be due to an inadequate sample size or confounding by mode of presentation. In our cohort, the frequency of root aneurysm is higher in L-R patients compared to both R-NC and TAV patients. Also, the OR for developing a root aneurysm is greater than 1.0 in L-R patients, which indicates an increased risk for this outcome when compared to TAV patients, and the OR is less than 1.0 for R-NC, which indicates a protective effect. While the increased outcome frequency for L-R phenotype patients, 14% vs. 9% for TAV and 8% for R-NC, and OR of 1.3 are not high enough to say that L-R patients are at a greater risk for root aneurysms, the incidence of a root aneurysm in the population is extremely low to begin with. So while a slight increase may not be statistically significant, clinically it may be important. Despite the lack of statistical significance, the frequencies presented in this study suggest
a need for L-R patients to be more closely monitored for root aneurysms versus their R-NC counterparts. For example, if a L-R patient undergoes surgery for their proximal ascending aneurysm (as most BAV patients eventually require), it may be reasonable to consider preemptively repairing the aortic root as well. If even a small amount of root dilatation is suspected, a surgeon can provide a graft for the aortic root to prevent a root aneurysm from ever occurring. This may not be considered for TAV or R-NC patients, but it may be viable for L-R patients, even if the incidence frequency is only slightly higher.

**Genetic link between BAV and aortic aneurysms**

The second portion of this study focused on attempting to find a genetic factor associated with aortic aneurysms and to determine if those factors were involved in BAV as well. The results of the GWAS provided us with multiple SNPs that could potentially be responsible for causing aneurysms. The results were separated by aneurysm phenotype and the top hits for each phenotype for identified. While the majority of SNPs were not associated with genes, we found multiple notable genes associated with each aneurysm phenotype. For proximal ascending aneurysms the genes were *IGF1R* (chromosome 15), *CDH13* (chromosome 16), *PARP4P2* (chromosome 13), *OR52B2* (chromosome 11), *TACC2* (chromosome 10), *EVL* (chromosome 14), *SLC30A8* (chromosome 8), and *WWOX* (chromosome 16). For root aneurysms the associated genes were *SFRP1* (chromosome 8), *GLB1L2* (chromosome 11), and *LRP1B* (chromosome
2). At first glance these none of these genes seem to have functions that relate specifically to the aorta. However, most of the genes listed above code for proteins that could have an effect of the cellular environment that could lead to aneurysm formation. A few of the genes listed above code for proteins that are involved in regulating apoptosis; one of which specifically regulates endothelial cell apoptosis and is associated with atherosclerosis. As previously mentioned, apoptosis in the medial layer of the aorta is one of the main pathological features of an aneurysm. Thus some of these genes could potentially be involved in causing the increased frequency of apoptosis in the aortic media that leads to aneurysms and should be inspected further in future research.

Another important detail about the results from the GWAS is the fact that both aneurysm phenotypes have different SNPs and genes involved. This supports the argument that there is a distinction between root and proximal ascending aneurysms and that classifying patients based on these two phenotypes would be beneficial on a clinical level. Being able to identify if patients are prone to a specific type of aneurysm based on their genes could be monumental in treating this potentially life-threatening illness. This is especially important because both root aneurysms and proximal ascending aneurysms require different treatment and care. If a physician can assess a patient’s risk of developing an aneurysm simply from their genetic information, he/she can tailor a preemptive course of treatment for the specific aneurysm phenotype (for which the patient is at risk) well before any symptoms arise.
While the GWAS provided us with multiple signals, after accounting for multiple comparisons, none of the results proved to be statistically significant at the GWAS level ($p<5*10^{-8}$). However, because of the low number of cases in each group, the power of test is not very high. So in fact these hit signals may be good candidate markers. In order to get validation of these results the study will be replicated in other cohorts.

Another important result from the GWAS was that multiple SNPs for aortic aneurysms (both phenotypes) matched SNPs previously found in BAV patients. However, these SNPs did not prove to be useful in linking a common genetic cause for aneurysms and BAV. All of the matching SNPs had weak statistical significance and some of them even showed a protective risk for aneurysm. Also the few matching SNPs belonged to genes whose function appears to have no correlation to either BAV or aortic aneurysms. For example, one of the SNPs that matched with BAV and root aneurysms belonged to the gene ACCSL on chromosome 11. This gene codes for an enzyme-like protein that may or may not have enzymatic activity. These results seem to suggest that there is no common genetic link between BAV and aortic aneurysms. This is not to say that there is no genetic cause of aneurysms, but its direct correlation to BAV does not appear to be genetic in nature. The genetic results indicate that there could be a genetic cause of aneurysm but it is unlikely to be caused by the same genes that cause BAV. This leads to the conclusion that, because a direct genetic link is not apparent, there must be, at least partly, a hemodynamic link between BAV and
aortic aneurysms. In all likelihood, the most likely association is a combination of the genetic factors discussed above and a hemodynamic effect caused by BAV. It is possible that hemodynamic stress caused by BAV could have an influence on the expression of the genes that cause aneurysms. Further studies are needed to first confirm the results of the GWAS and then to determine if BAV has any effect on the expression on the genes shown to cause aneurysms.

Limitations

This study has limitations that may have had an effect on the results. The patients in the study were all cardiac surgery patients, most of whom were having surgery for valve replacement or aneurysm repair. Thus this could have had a large influence on the reported frequencies of aneurysms based on valve type, especially for TAV patients. Because the patients were all surgical candidates, frequency of aneurysms identified in them could be higher than normally expected. Almost all BAV patients eventually require aortic surgery so this may not have a major impact on those patient frequencies; however, the aneurysm incidence for patients with a normal aortic valve may have been over estimated in this study. This would have had an effect on the statistical analysis and thus may have caused certain data to appear insignificant. Also in this regard, the limited number of patients may also have had an effect. Because the cohort was small in size, the results may have been statistically insignificant. With a larger patient base, the statistical outcome may have been different. The small size
patient size may have had also had an effect on the GWAS results. None of the SNPs were considered statistically significant at the GWAS level; however, some were close. If the patient population had been larger, the statistical analysis may have been slightly different and some of the SNPs may have had a more significant P value. In order to account for these limitations, the study should be repeated with a larger patient population and a control group that is more representative of the normal population.

Another potential limitation is fact not all of the data was arbitrated by us personally. A portion of the data came from surgical reports that may not have been entirely accurate. Because much of the imaging was missing or not available for these patients, we could not arbitrate all of the data ourselves. Thus it was necessary for us to get this data from a source that we believed to be reliable. This does not mean the data from the surgical notes is not accurate; however, because we did not arbitrate it personally, we cannot truly be 100% sure. It is possible that errors in the data could have had an effect on the results of our study. Any future researchers that replicate our study should make every attempt to arbitrate all their data themselves.

**Conclusion**

In conclusion, the current study has provided useful information to better understand BAV, aortic aneurysms, and the relationship between the two disorders. The limitations presented above have made it so that the results of the
study may not be entirely conclusive; however, they provide a good starting point for future studies. The unexpected results of the non-genetic portion were clear enough to suggest that the classification system used above can provide clinical benefit for both BAV and aneurysm patients. The results of the GWAS have provided potentially good markers that could be pursued in investigations of how BAV leads to aneurysms and how to better treat them. The results need to be replicated in a larger cohort and one day they could prove to be helpful in the clinical setting.
REFERENCES


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• Department of Anesthesiology
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• Research Assistant

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• Miriam Hospital – Brown University
• Providence, RI
• P.A.Q.S. Project
• Behavioral research study aimed at helping parents of asthmatic children quit smoking
• Performed participant recruitment, participant screening, and data entry

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• Providence, RI
• A.B.L.E. Project
• Behavioral research study aimed at helping handicapped people quit smoking
• Performed participant recruitment, participant screening, and data entry

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