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## The Genetic Basis of Turner Syndrome Aortopathy

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### Abstract

Our goal is to identify the genetic underpinnings of bicuspid aortic valve and aortopathy in Turner syndrome. We performed whole exome sequencing on 188 Turner syndrome study subjects from the GenTAC registry. A gene-based burden test, SKAT-O, was used to evaluate the data using bicuspid aortic valve (BAV) and aortic dimension z-scores as covariates. This revealed that *TIMP3* was associated with BAV and increased aortic dimensions at exome-wide significance. It had been previously shown that genes on chromosome Xp contribute to aortopathy when hemizygous. Our analysis of Xp genes revealed that hemizygosity for *TIMP1*, a functionally redundant paralogue of *TIMP3*, increased the odds of having BAV aortopathy compared to individuals with more than one *TIMP1* copy. The combinatorial effect of a single copy of *TIMP1* and *TIMP3* risk alleles synergistically increased the risk for BAV aortopathy to nearly 13-fold. *TIMP1* and *TIMP3* are tissue inhibitors of matrix metalloproteinases (TIMPs) which are involved in development of the aortic valve and protection from thoracic aneurysms. We propose that the combination of *TIMP1* haploinsufficiency and deleterious variants in *TIMP3* significantly increases the risk of BAV aortopathy in Turner syndrome, and suggest that *TIMP1* hemizygosity may play a role in euploid male aortic disease.

### Keywords

Aortic disease; Bicuspid aortic valve; Congenital cardiovascular disease; Polygenic trait

The goal of this project is to identify the genetic basis of bicuspid aortic valve (BAV) and associated aortopathy in Turner syndrome. Turner syndrome is an aneuploid condition resulting from the complete or partial loss of the second sex chromosome. The resulting deficiency of sex chromosome genes predisposes individuals with Turner syndrome to diseases with a sex bias in the general population, including BAV. BAV is the most common cardiovascular birth defect in humans, affecting about 2 to 3 percent of euploid males and around 0.05 percent of euploid females, whereas it affects 25–30% of individuals born with Turner syndrome, which is a 50–100 fold increase over the euploid population. The high

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Conflict of Interest

The authors do not have any conflicts of interest.

incidence in Turner syndrome and the substantial sex bias towards males in the euploid population suggests that there is protection conferred by having a second X chromosome.

Our study was designed to determine the genetic basis of BAV in Turner syndrome with the presumption that there are contributions from autosomal gene variants in addition to an X chromosome component. Importantly, about 95 percent of individuals with Turner syndrome who have an aortic dissection also have BAV. So there is a clear association between BAV and later onset aortopathy in Turner syndrome. In addition to identifying genes associated with BAV, we also want to find the genetic intersection between BAV and aortopathy.

We use the disease threshold model shown in figure 1 to provide a framework for thinking about how BAV in Turner syndrome may occur. In the general euploid population an unknown number of genetic variants and/or other risk factors must be present in order to breach the theoretical threshold of disease. On the other hand, individuals with Turner syndrome are genetically predisposed to having BAV aortopathy as the result of having a complete or partial loss of the second sex chromosome. The sex chromosome gene deficiency is not alone sufficient to push individuals with Turner syndrome above the disease threshold, but we hypothesized that it will take few additional risk factors for them to manifest the disease. In fact, one additional risk factor may suffice.

We tested this hypothesis using a whole exome sequencing study of individuals from the GenTAC registry/ Turner syndrome cohort, with and without BAV. (Eagle & GenTAC Investigators, 2009; Kroner et al., 2011) We used BAV status and aortic Z-scores as the phenotypic variables for the analysis (Corbitt, Maslen, Prakash, Morris, & Silberbach, 2018; Quezada, Lapidus, Shaughnessy, Chen, & Silberbach, 2015), with aortic enlargement with a Z-score of greater than 2 as a proxy for aortic aneurysm formation. The whole exome sequencing on 188 Turner syndrome samples was done by the NHLBI Resequencing and Genotyping Service at the University of Washington.

We used a sequence kernel associated test, or SKAT-O to analyze the data. (Wu et al., 2011) In SKAT-O, which is a gene burden by phenotype association test, genetic variants are weighted based on allele frequency as a proxy for potential deleteriousness and clustered into genes for an association analysis. The power to detect a signal is increased because the multiple testing burden is based on the number of genes tested instead of the traditional single variant by phenotype testing where every variant is an individual test. Figure 2 shows the Manhattan plots resulting from the SKAT-O analyses. Figure 2A is the result using BAV as the variable. (Corbitt, Morris, et al., 2018) A single gene, *TIMP3*, reached exome-wide significance. Adding in aortic root dimensions or ascending aortic dimensions as continuous covariates, we found exome wide significance with BAV and aortic root Z-scores, where the *TIMP3* signal actually rose in significance, indicating that it is associated with both BAV and aortic dilation (Figure 2B). The associated Q-Q plots are shown in figure 3.

We analyzed the variants identified in *TIMP3* to determine what was driving the association. There were a total of four variants identified in all of the exomes, so a small amount of variation is tolerated in this gene. We found that one variant in particular, rs11547635, which is a synonymous single nucleotide variant (SNP) in the codon for amino acid position at

p.Ser87, was the significant driver of association here. It was present at a higher than expected frequency in cases compared to controls, and it was also predicted to be highly deleterious. We used CADD scores as a measure of the degree of deleteriousness, with a score of >15 indicating that a variant is in the top 5% of most deleterious variants in the genome(Kircher et al., 2014). All of the variants and their allele frequencies are shown in Table 1.

Two other variants also contributed to the association. They were both very rare, observed only in cases. We also observed that the p.Ser87 variant is always accompanied by a synonymous SNP at the codon for amino acid position 83, p.His83, which appears to have functional significance.

We wished to replicate the *TIMP3* association, which we were able to do thanks to Dr. Claus Gravholt, who contributed samples from his Danish Turner syndrome registry. We did a SKAT-O analysis after sequencing the exons of *TIMP3* and found that the association replicated with the p.Ser87 position still acting as the driver, as shown in Table 2, which was statistically significant even though it was a small replication cohort. And we also saw the co-segregation with p.His83, and one of the very rare variants, also only in cases.

Based on this association we are interested in understanding the functional consequences of these *TIMP3* variants. We know from the cancer literature that the linked p.His83 and p.Ser87 SNPs are associated with reduced levels of TIMP3 in plasma.(Bashash et al., 2013; Jackson, Defamie, Waterhouse, & Khokha, 2017; Su et al., 2015) Our preliminary data from western blot analysis of recombinant TIMP3 from constructs harboring the p.His83 and p.Ser87 SNPs, together and separately appears to support this association (Figure 4). Expression constructs were made that carried each of the variants alone, and together. Western blot analysis of protein secreted from HEK293 cells transfected with the various *TIMP3* constructs shows an overall reduction in secreted TIMP3 when pHis83= and/or p.Ser87= are present, particularly of the glycosylated form. Consequently, we propose that a reduction in extracellular TIMP3 is the biologically relevant mechanism of action for these SNPs, which is important in maintaining structural integrity of the aorta, as TIMP3 is a member of the tissue inhibitor of matrix metalloproteinase family. Matrix metalloproteinases (MMPs) are enzymes that are known to break down extracellular matrix. MMP activity is controlled by TIMPs through direct binding, which is critical to maintaining tissue homeostasis.

TIMP3 is known to control MMPs 2, 3, 7, 9, 13, and 14. Significantly, MMPs 2 and 9 are the most highly expressed MMPs in thoracic aortic aneurysms, and an imbalance between TIMPs and MMPs is thought to be the underlying pathogenic mechanism in thoracic aortic aneurysms and progression to dissection.(Abaci, Kocas, Kilickesmez, Uner, & Kucukoglu, 2013; Allaire, Forough, Clowes, Starcher, & Clowes, 1998; Ikonomidis et al., 2004; Koullias, Ravichandran, Korkolis, Rimm, & Elefteriades, 2004; Li et al., 2010; Rabkin, 2014; Zhang, Shen, & LeMaire, 2009; Zhang et al., 2014) This occurs when increased MMP activity in the tunica media of the aorta disrupts the usually highly ordered elastin-based extracellular matrix. As the matrix is degraded, macrophages are allowed to infiltrate, and aneurysms form.

Going back to our disease threshold model (Figure 1), we're proposing that the p.His83 and p.Ser87 variants in *TIMP3* exist as common variants in the general population and are presumably benign unless triggered by another genetic event where they can become pathogenic. In particular, these variants are associated with progression of certain cancers. In Turner syndrome we propose that there is a pathogenic interaction between these *TIMP3* variants and hemizyosity of a gene or genes on the X chromosome that is the underlying etiology for BAV aortopathy in that population. To address this, we did a bioinformatic analysis to identify candidate genes. We know from prior publications that the region of the X chromosome associated with BAV and aortic aneurysms is actually the short arm, Xp. (Bondy et al., 2013; Prakash et al., 2016) We established inclusion criteria based on the premise that reduced dosage of a gene or genes on Xp increase risk of BAV aortopathy. There were three criteria to consider. One, the gene must be unique to the X chromosome. There can't be a homolog elsewhere in the genome based on our premise that it's a reduction in dosage that is involved, specifically on the Y chromosome. The gene also must escape X inactivation, so the gene on the second X chromosome has the capacity to be expressed. Finally, any candidate gene needs to be expressed in the developing aortic valve to be involved with bicuspid aortic valve and in the mature aorta to be associated with later onset aortopathy.

In our analysis, out of the 247 Xp genes, only 3 met those criteria. And they are *UBA1*, *RBM3*, and *TIMP1*. *UBA1* is associated in mouse models and in humans with spinal and muscular atrophy, and there is no cardiovascular phenotype. Likewise, *RBM3* is associated with neurodegenerative disease both in mouse models and in humans. But once again, there is no cardiovascular phenotype, even though both are expressed at low levels in the aorta. On the other hand, reduced expression of *TIMP1* is associated in humans in thoracic aortic aneurysms and in mouse models where the *TIMP1*-deficient mouse is susceptible to developing aortic aneurysms. *TIMP1* is expressed in the aorta (Akahane, Akahane, Shah, & Thorgeirsson, 2004), but also in the developing aortic valve (Dreger, Taylor, Allen, & Yacoub, 2002), and its expression level is tenfold higher than *UBA1* or *RBM3*. Consequently, we propose that *TIMP1* is the most likely Xp gene to be involved in BAV aortopathy. Importantly, *TIMP1* and *TIMP3* are functionally redundant in the aorta, and both control MMPs 2 and 9, which are known to be upregulated in thoracic aortic aneurysms, so we consider this association to be functionally very important.

Next we asked the question, is *TIMP1* copy number associated with aortic disease and, most importantly, bicuspid aortic valve? We were able to do this because Turner syndrome is not always monosomy X, but in some cases there is just a partial loss of the second sex chromosome, and our GenTAC cohort reflected this diversity. About 50 percent have a complete monosomy X. Around 30 percent have a partial deletion or rearrangement of a second X chromosome. Twenty percent are mosaic for a second sex chromosome, and a few have some residual Y chromosome material. In addition, since there is no Y chromosome *TIMP1* homologue males only have a single copy of *TIMP1*.

We used our whole exome data to construct molecular karyotypes for our entire cohort so that we could determine the copy number of *TIMP1*, including accounting for mosaicism where some cells may have one copy of *TIMP1* and other may have two copies. For

example, if 50 percent of cells are 45,XO, and 50 percent are 46,XX, then the average copy number would be 1.5, which would be expected to result in a higher average plasma protein level as it is a secreted protein. When we did the association study, we showed that *TIMP1* copy number is associated with bicuspid aortic valve, as shown in figure 5. Having only one copy of *TIMP1* confers a greater than fourfold increased risk for having a bicuspid aortic valve compared to more than one copy of *TIMP1*.

As expected, *TIMP1* expression is reflective of copy number as the second copy is known to polymorphically escape X inactivation.(Anderson & Brown, 1999) Figure 6 shows *TIMP1* protein levels in plasma from individuals in our Turner syndrome cohort. The expression level associated with a single copy of *TIMP1* is consistently low. However, when there is more than one copy, the expression level is generally increased but is also highly variable. This is based on the fact that *TIMP1* polymorphically escapes X inactivation in humans, and expression levels are significantly different.

We then determined if there was a combinatorial effect between *TIMP1* copy number and the *TIMP3* risk alleles. As shown in Table 3, there is a synergistic effect of having both the *TIMP3* risk alleles and only one copy of *TIMP1*, with a nearly 13-fold increase in risk for BAV and aortic root dilation compared to having more than one copy of *TIMP1* and no *TIMP3* risk allele. Figure 7 shows the influence of *TIMP1* copy number on aortic dimensions. The mean aortic root Z-score is significantly greater in individuals with only one copy of *TIMP1*, compared to those with greater than one *TIMP1* copy number (Figure 7A). Likewise, the mean ascending aorta Z-score is significantly larger with only one copy of *TIMP1* (Figure 7B).

And finally, one more piece of data that we find very compelling, and this was done by Drs. Michael Silberbach and Hal Dietz looking at a Turner syndrome aorta that was donated to Dr. Silberbach from a young woman with Turner syndrome who died from a type B aortic dissection. The pathology is shown in Figure 8. Panels A and C show an aortic section from a normal sex- and age-related control. Panels B and D are from the aorta of the Turner syndrome individual. The aorta section from the Turner syndrome patient was from non-aneurysmal tissue. The aortas from panels A and B are stained with an antibody against connective tissue growth factor (CTGF). The staining shows that CTGF expression is greatly increased in the Turner syndrome aorta compared to the normal control. Panels C and D are stained with trichrome, which shows a significant amount of fibrosis compared to the normal control, likely as a result of the increase in CTGF expression.

Figure 9 shows the same aortas immunohistochemically stained to detect SMAD4 as a proxy for TGF-beta signaling. Panels A and C are from the control aorta, and panels B and D are the Turner syndrome aorta. Turner syndrome aorta shows an increased level of SMAD4 expression compared to the normal control, indicating that there is an increase in TGF- $\beta$  signaling. This is a familiar scenario in genetically-triggered thoracic aortic aneurysms. An increase in TGF- $\beta$  signaling was originally noted in aneurysmal tissue from the aortas on individuals with Marfan syndrome.(Neptune et al., 2003) Although the specific significance of this process is still under investigation, it gives a framework for developing a pathogenic model for the development of thoracic aortic aneurysms in Turner syndrome, as shown in

Figure 10. Our data indicates that in individuals with Turner syndrome there can be an inherent imbalance in TIMPs and MMPs due to hemizyosity of *TIMP1*, which is further exacerbated in the presence of *TIMP3* risk alleles. As a result, there is a loss of inhibition of MMPs 2 and 9 which proteolytically degrades the extracellular matrix (ECM) of the aortic wall. Degradation of the ECM releases active TGF- $\beta$ , which is held in its latent inactive form in the ECM under conditions of tissue homeostasis. As TGF- $\beta$  activity increases there is a process by which fibrosis and inflammation occurs, including an increase in MMP activity, eventually resulting in an aortic aneurysm.

We can now speculate that the sex bias that predisposes euploid males to bicuspid valve aortopathy may, in part, be due to a relative impairment of TIMP1/MMP signaling pathways triggered by TIMP1 hemizyosity in males.

In conclusion, we've shown based on whole exome sequencing that deleterious variants in *TIMP3* are associated with BAV and aortic enlargement and that *TIMP1* is likely the Xp chromosome sensitizing factor for BAV and aortopathy in Turner syndrome. We propose that during valve morphogenesis inherent reductions in *TIMP1* copy number and SNP-associated *TIMP3* expression increase risk for BAV. In the mature aorta, *TIMP1* deficiency due to hemizyosity coupled with reduced TIMP3 expression sensitizes the aortic wall, leading to aortopathy.

## Acknowledgments

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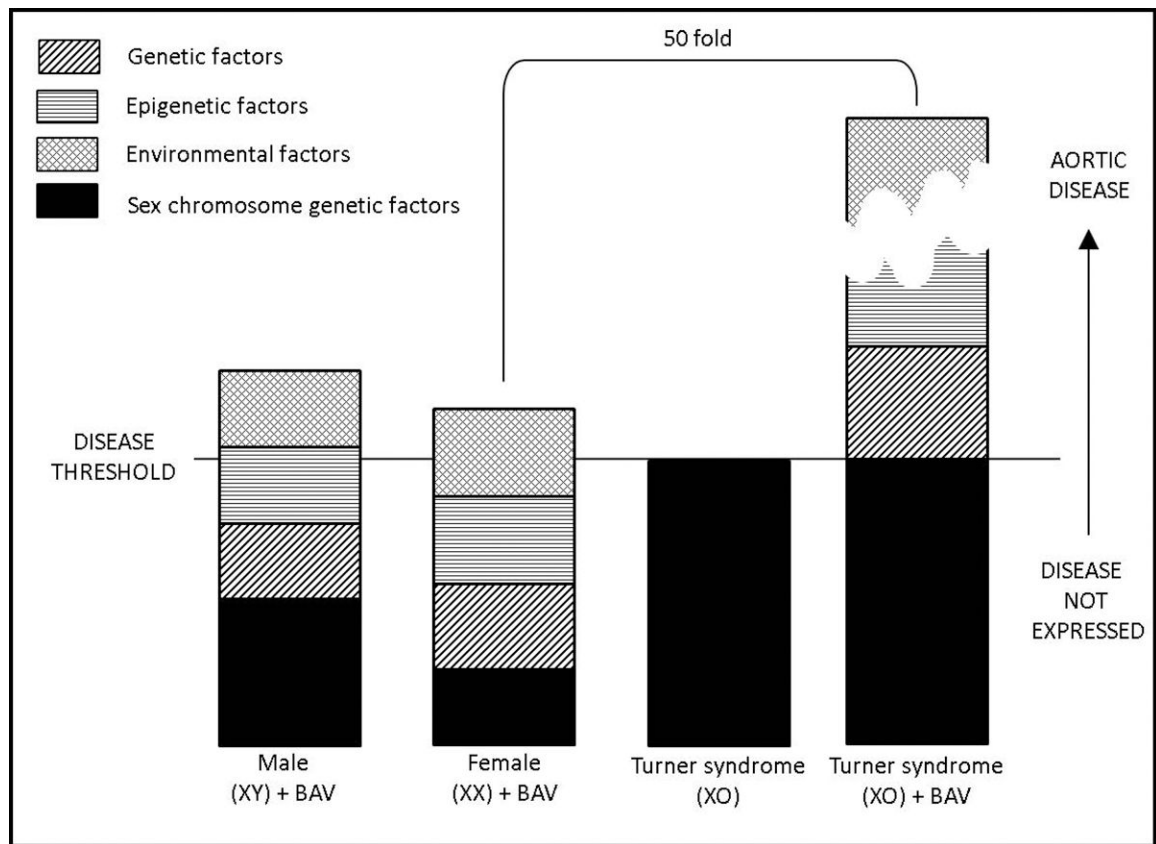
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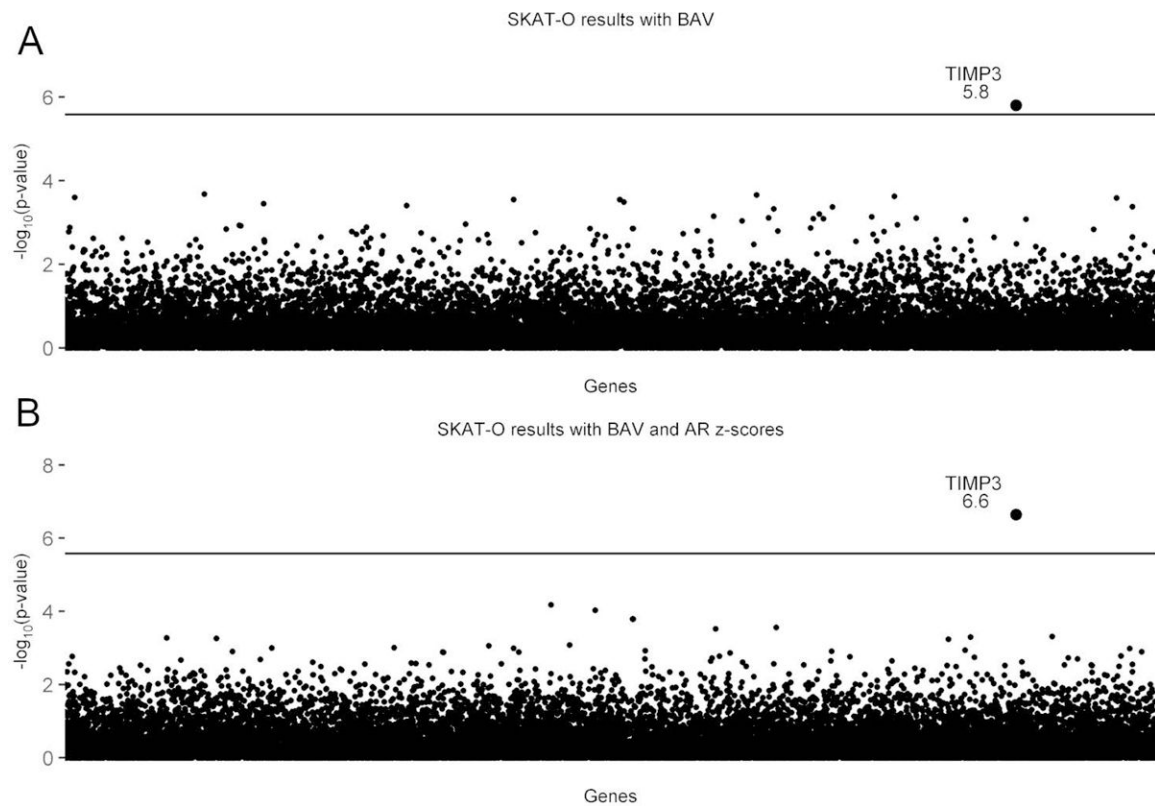
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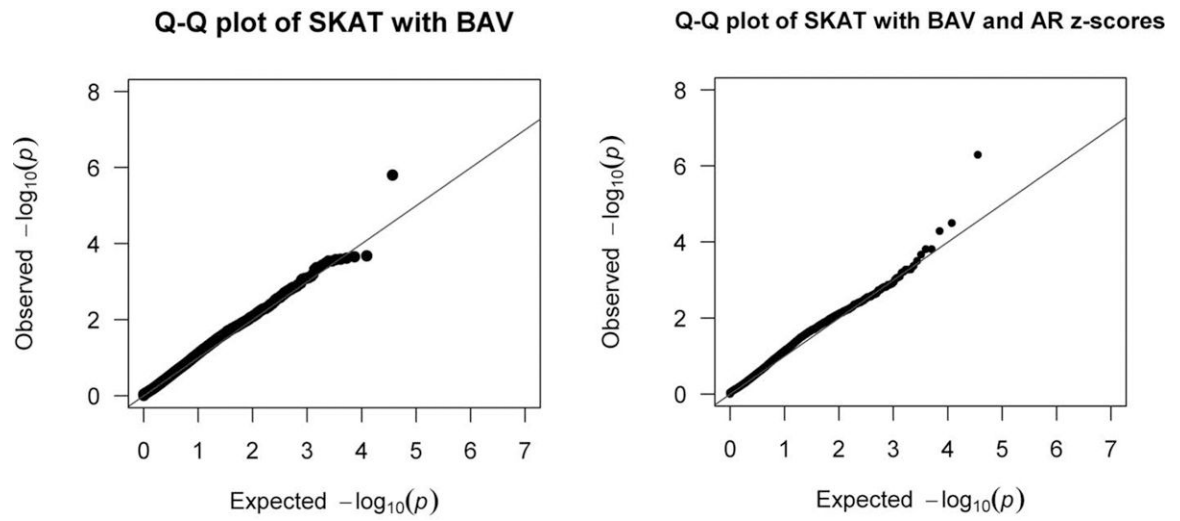
**Figure 1. Disease threshold model to illustrate polygenic disease and the role of effect size of risk variants.**

The horizontal dotted line represents a theoretical threshold of disease. Factors, such as genetic variants are represented by boxes forming vertical columns. Columns that cross the dotted line breach the disease threshold indicating that a sufficient burden of risk factors exists to cause the disease, in this case BAV. In Turner syndrome the loss of the second sex chromosome is a risk factor of very large effect size. However, it is not sufficient alone to breach the disease threshold for BAV as not everyone with Turner syndrome has BAV. However, it is likely that fewer risk factors are required to cause BAV compared to the euploid population, which has an inherently low risk.



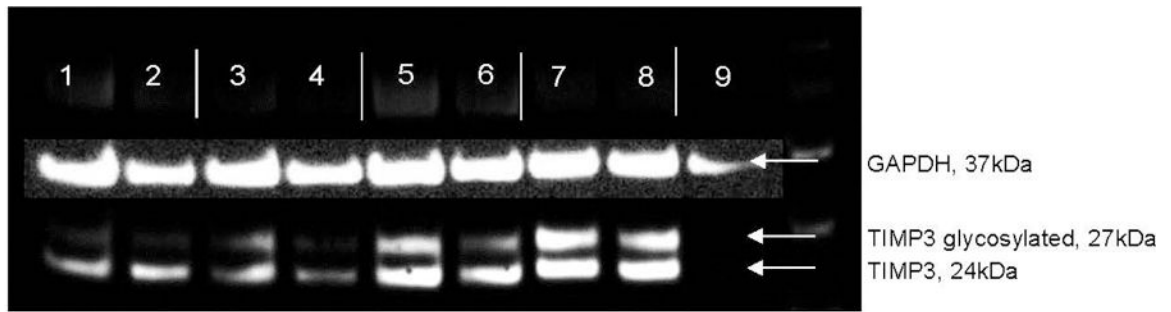
**Figure 2. SKAT-O Analysis Shows that *TIMP3* Variants are Associated with BAV/TAD.**

Manhattan plots showing the exome-wide significant finding that *TIMP3* variants are associated with BAV, and with AR enlargement as an indicator of TAD. The horizontal line is the threshold for exome-wide significance (based on testing 19,392 genes, the exome-wide significance  $p\text{-value}=2.578\times 10^{-6}$ ). *TIMP3* is the only gene that exceeds exome-wide significance. It is notable that no other genes approach the significance line. A) Shows the association with BAV as the sole predictor ( $p=1.58\times 10^{-6}$ ). B) Shows the association results for BAV and aortic root (AR) z-scores as covariates ( $p=2.27\times 10^{-7}$ ). The significance level for *TIMP3* increases nearly 10-fold when AR z-scores were added. (From Corbitt H et al. (2018) *TIMP3* and *TIMP1* are risk genes for bicuspid aortic valve and aortopathy in Turner syndrome. PLoS Genetics, 2018 Oct 3;14(10):e1007692. doi: 10.1371/journal.pgen.1007692.)



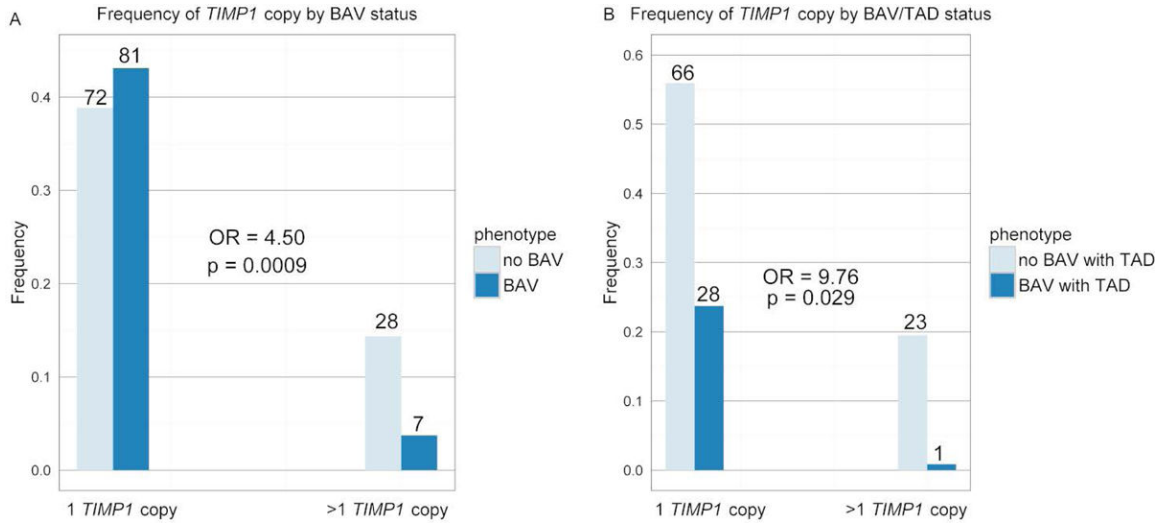
**Figure 3. Quantile-Quantile (Q-Q) Plots for the SKAT-O Analyses.**

Q-Q plots for SKAT-O analysis of BAV and BAV with AR Z-scores, which shows no significant deviation from the normal distribution.

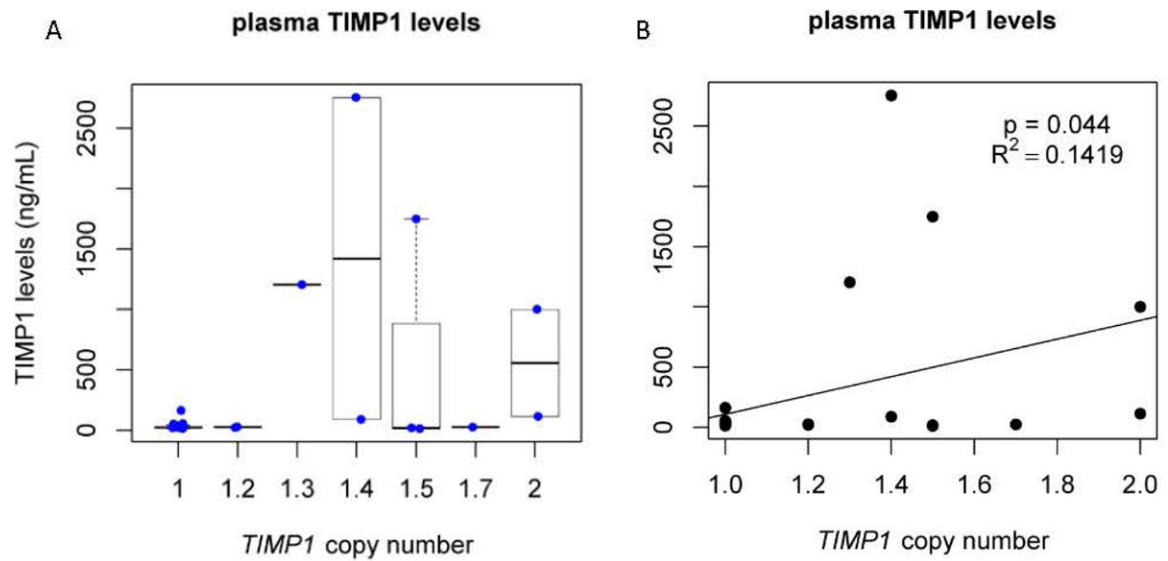


**Figure 4. *TIMP3* variants decrease expression.**

Western blot of secreted protein from *TIMP3* constructs, in duplicates. Lanes 1–2 His83/Ser87 double variant; Lanes 3–4, His83; Lanes 5–6 Ser87; Lanes 7–8 wild type; Lane 9, empty vector. GAPDH loading control is superimposed. Note that the His83 and Ser87 variants significantly reduce secreted *TIMP3* expression, particularly the glycosylated form.



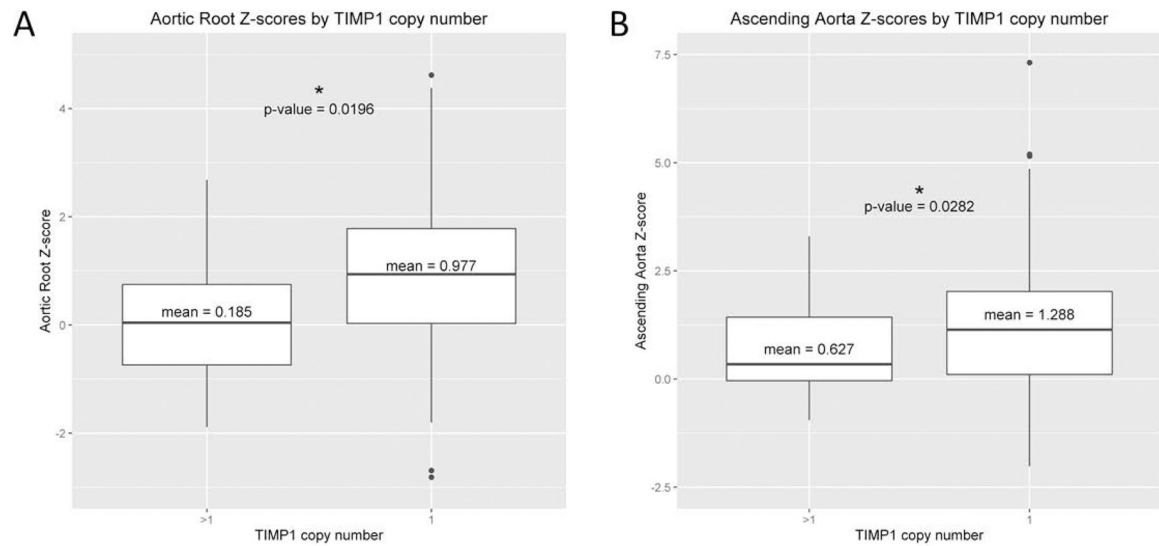
**Figure 5. *TIMP1* copy number is associated with the risk of BAV and BAV with TAD.** Bar graph showing the frequency of BAV with or without TAD when only one copy of *TIMP1* is present compared to the frequency when there is more than one copy of *TIMP1*. **A)** The dark blue bars represent individuals with a diagnosis of BAV and the light blue bars represent individuals without a BAV. **B)** The dark blue bars represent individuals with a diagnosis of BAV with TAD and the light blue bars represent individuals without a BAV with TAD. For both a logistic regression model was run, with *TIMP1* copy number as the categorical predictor and the phenotype as the response variable. Odds ratio and p-value were calculated and showed that having a single copy of *TIMP1* significantly increased the odds of having a BAV and a BAV with TAD. (From Corbitt H et al. (2018) *TIMP3* and *TIMP1* are risk genes for bicuspid aortic valve and aortopathy in Turner syndrome. PLoS Genetics, 2018 Oct 3;14(10):e1007692. doi: 10.1371/journal.pgen.1007692.)



**Figure 6. TIMP1 protein levels in plasma.**

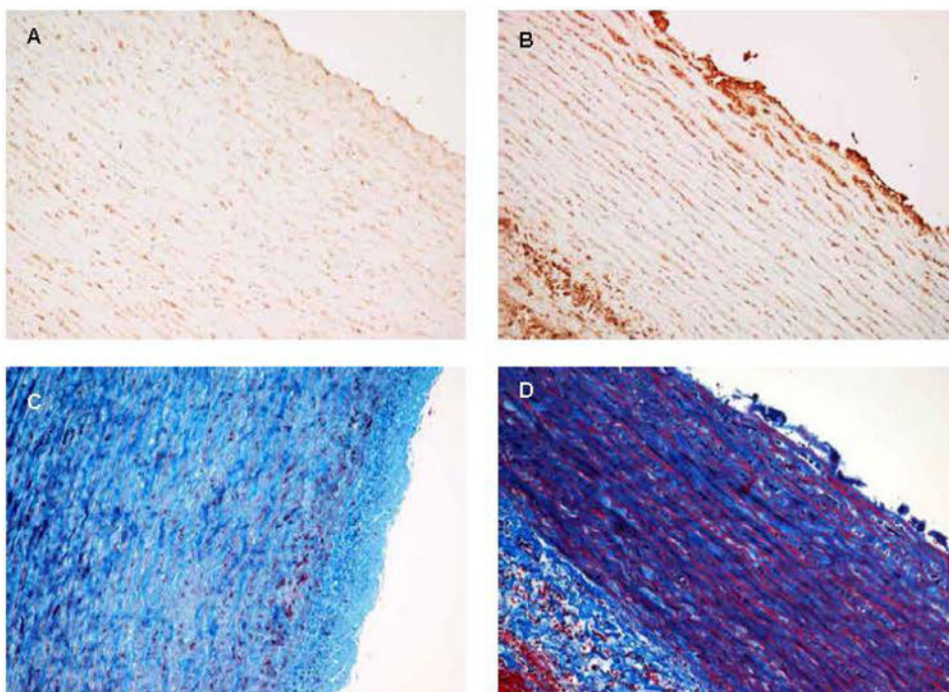
Expression of TIMP1 levels in plasma as measured by ELISA plotted by the number of *TIMP1* gene copy number. Note that *TIMP1* copy number is significantly correlated with TIMP1 protein levels in plasma. The samples with only one copy of *TIMP1* are tightly clustered with little variability, whereas the samples with more than 1 copy are highly variable, as has been previously described.





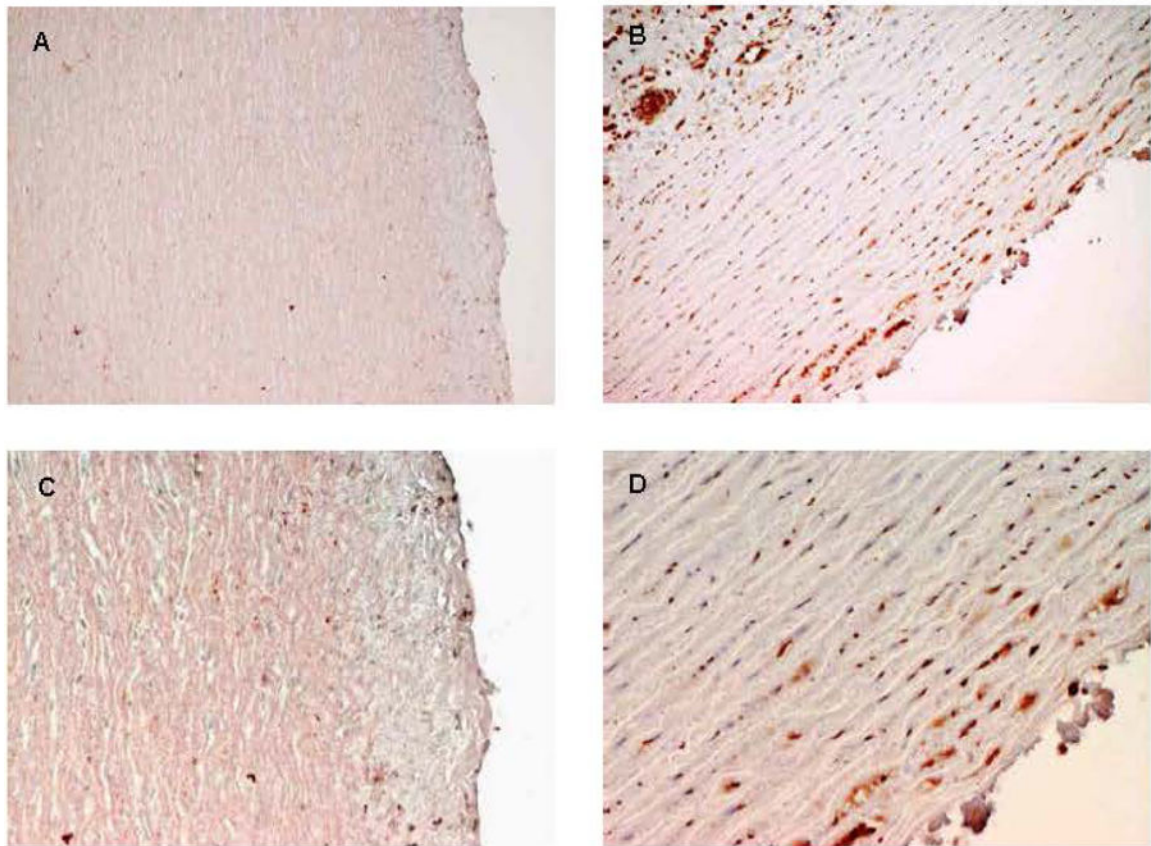
**Figure 7. *TIMP1* copy number vs aortic z-scores.**

Panel A shows a box plot representing study subject aortic root z-scores plotted against *TIMP1* copy number. Panel B shows the box plot for ascending aorta z-scores plotted against *TIMP1* copy number. Note that for both aortic root and ascending aorta z-scores there is a significant difference in aortic diameters comparing individuals with only one copy of *TIMP1* to those with greater than one copy of *TIMP1*.



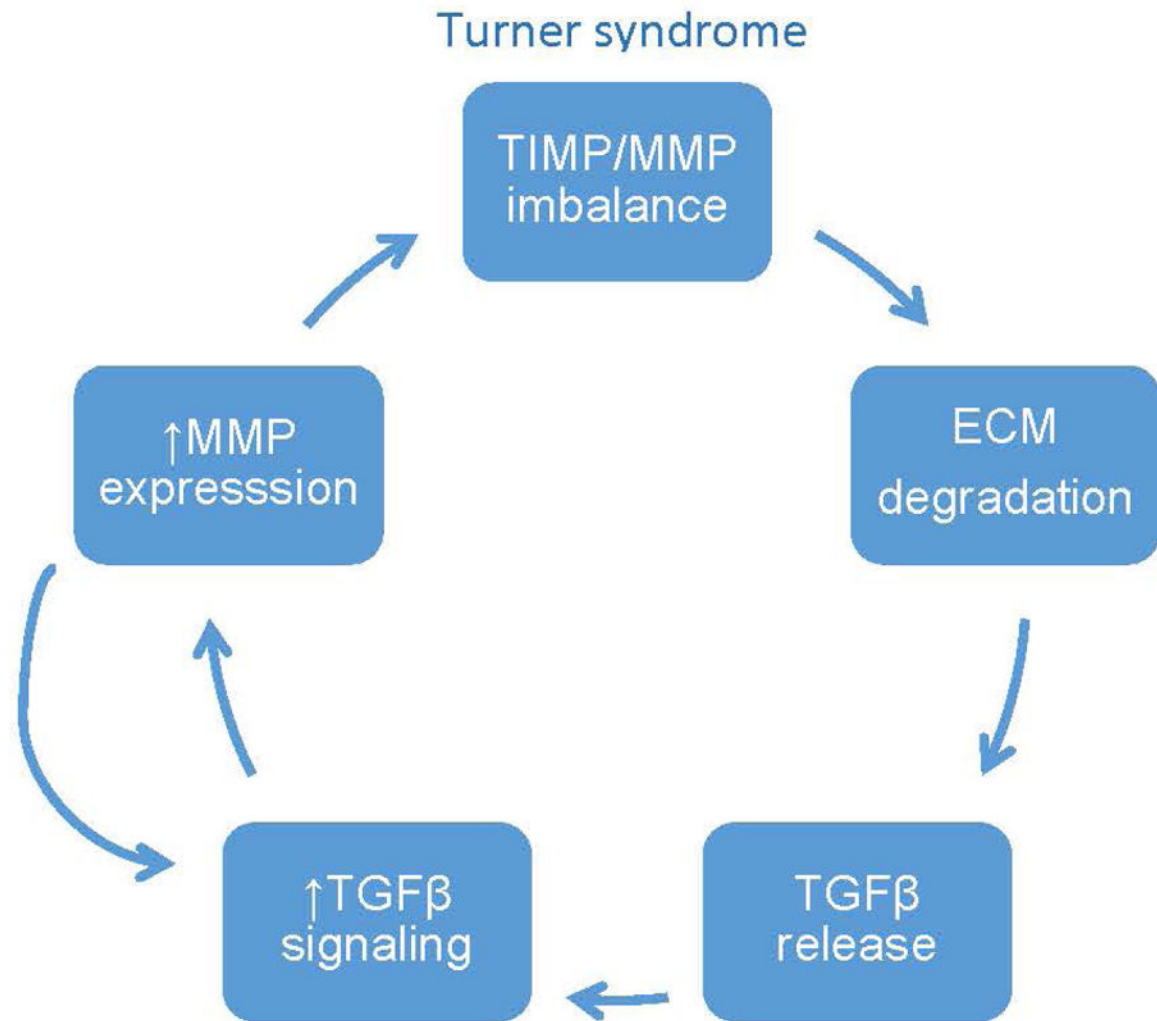
**Figure 8. TS aorta CTGF and extracellular matrix staining.**

Analysis of the same aortic tissue from a young woman with TS who died from a dissection of the ascending aorta to visualize extracellular matrix components. The top two panels (A, B) are stained with an antibody to connective tissue growth factor (CTGF). Panel A is from an age and gender matched normal control aorta. Note the lack of staining for CTGF. Panel B is from a non-aneurysm section of aorta from the TS patient. Note the significantly increased level of CTGF indicating an activation of connective tissue production. Panels C and D are stained with trichrome, staining collagens blue. Note the substantial increase in staining in the TS aorta (D) compared to the control (C), showing increased collagen deposition in the TS aorta (fibrosis), which is thought to be compensatory for aortic wall instability.



**Figure 9. TS aorta SMAD staining.**

Immunohistochemical analysis the same aortas shown in figure 6 to assess levels of TGF- $\beta$  signaling. The two left panels (A, C) are from control aortas. The two right panels are sections from the TS aorta. All are stained with p-SMAD antibody. SMADs are downstream products of TGF- $\beta$  signaling. Note the increase in staining (reddish-brown) in the TS aorta (Panels B and D) compared to a normal control (Panels A and C), indicating an increase in p-SMAD production as a result of increased TGF- $\beta$  signaling. Excessive TGF- $\beta$  signaling is seen in aortas from individuals with Marfan syndrome and is thought to contribute to aortic wall instability. This is the first demonstration of excessive TGF- $\beta$  signaling in TS.



**Figure 10. Theoretical model of aortic disease pathogenesis in TS.**

This model depicts the proposed pathogenic events underlying aortopathy in Turner syndrome. We propose that there is an inherent imbalance in the TIMP/MMP ratio that leads to the degradation of the ECM, including fibrillin-1 microfibrils. As in Marfan syndrome this releases active TGF $\beta$  resulting in an increase in TGF $\beta$  signaling. One of the consequences of TGF $\beta$  signaling is an increase in MMP expression, which feeds into the TIMP/MMP imbalance. There is also a feedback loop through which increased MMP activity proteolytically cleaves latent TGF $\beta$  producing the active form.

**Table 1.**  
**Summary of the *TIMP3* Variants Associated with BAV/TAD<sup>6</sup>**

(From Corbitt H et al. (2018) *TIMP3* and *TIMP1* are risk genes for bicuspid aortic valve and aortopathy in Turner syndrome. PLoS Genetics, 2018 Oct 3;14(10):e1007692. doi: 10.1371/journal.pgen.1007692.)

rsID	Protein Change	CADD Score	Expected Allele Frequency (Alleles/Total)	Observed Allele Frequency in Cases (Cases/Total)	Observed Allele Frequency in Cases (Alleles/Total)	Observed Allele Frequency in Controls (Controls/Total)	Observed Allele Frequency in Controls (Alleles/Total)
rs9862	p.His83=	2.597	49.1% (32790/66734)	64.8% (57/88)	43.8% (77/176)	71.0% (71/100)	50.5% (101/200)
rs11547635	p.Ser87=	16.67	7.1% (4735/66738)	23.8% (21/88)	11.9% (21/176)	6.0% (6/100)	3.5% (6/200)
rs149161075	p.Phe172=	5.801	0.3% (203/66736)	2.3% (2/88)	1.1% (2/176)	0% (0/100)	0% (0/200)
rs369072080	p.Gly173=	0.002	0% (0/66738)	1.1% (1/99)	0.6% (1/176)	0% (0/100)	0% (0/200)

All of the *TIMP3* variants identified through whole exome sequencing of subjects in our Turner syndrome cohort are listed. The dbSNP rs identifier is listed, along with the consequence of the change, ExAC expected allele frequencies (European non-Finnish), CADD score, the number of subjects that had a BAV (case) or a normal valve (control), and the allele frequency of each variant, reported as percentages.

**Table 2.**  
**Replication Cohort Sequencing Results<sup>6</sup>**

(From Corbitt H et al. (2018) *TIMP3* and *TIMP1* are risk genes for bicuspid aortic valve and aortopathy in Turner syndrome. PLoS Genetics, 2018 Oct 3;14(10):e1007692. doi: 10.1371/journal.pgen.1007692.)

Chromosome	Position	ID	REF	ALT	MAF cases	MAF controls
22	33253280	rs9862	T	C	50.00%	50.00%
<b>22</b>	<b>33253292</b>	<b>rs11547635</b>	<b>C</b>	<b>T</b>	<b>14.29%</b>	<b>6.40%</b>
22	33255244	rs149161075	C	T	3.57%	0.00%

Gene	P value	N variants in Test
<i>TIMP3</i>	0.03734*	3

Variants identified in Danish cohort by Sanger sequencing of *TIMP3* exons.

ID; rs identifier from dbSNP

MAF; minor allele frequency

P value; calculated by SKAT-O gene-based association test

\* reaches the significance threshold of <0.05

N; number

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**Table 3.**  
**Combinatorial Effects of *TIMP1* Copy Number and *TIMP3* SNP rs11547635 on the Outcome of BAV or BAV with TAD<sup>6</sup>**

(From Corbitt H et al. (2018) *TIMP3* and *TIMP1* are risk genes for bicuspid aortic valve and aortopathy in Turner syndrome. PLoS Genetics, 2018 Oct 3;14(10):e1007692. doi: 10.1371/journal.pgen.1007692.)

Groups, outcome of BAV with TAD	N	N affected	% affected	OR	95% CI	P value
no rs11547635 & >1 <i>TIMP1</i> copy	22	2	9.09%	1.00 <sup>**</sup>	-	-
no rs11547635 & only 1 <i>TIMP1</i> copy	78	20	25.64%	3.45	0.89 – 22.80	0.115
yes rs11547635 & >1 <i>TIMP1</i> copy <sup>*</sup>	2	0	0.00%	-	-	-
yes rs11547635 & only 1 <i>TIMP1</i> copy	16	9	56.25%	12.86	2.57 – 99.39	0.004

Logistic regression model for the combinatorial effect of have both rs11547635 and one copy of *TIMP1* on the outcome of having a BAV with TAD.

BAV and TAD are defined as having a BAV and at least one z-score  $\geq 1.9$  (rounded to the 10th decimal place); those without an AR or AAO measurement were excluded.

<sup>\*</sup> For this group, the sample size was too small, so an accurate odds ratio could not be calculated.

N; total number of subjects.

N affected; number of subjects with BAV or BAV and TAD.

<sup>\*\*</sup> Reference group