The Power of Collaboration: Institutional Partnership Initiatives for Data Sharing Leads to *TNXB* Phenotype Expansion

Arpita Neogi, MS, CGC[1]; Meghan Towne, MS, CGC[2]; Arya Mani, MD [1,3]

[1]Cardiovascular Medicine, Yale University; [2]Ambry Genetics; [3]Department of Genetics, Yale University

Background

TNXB Gene

- Associated with autosomal recessive classical-like Ehlers Danlos Syndrome Type 1 (clEDS)
- Characterized by joint hypermobility, velvety skin, and hyperextensible skin
- Identified in a case report in individuals with abdominal and iliac artery aneurysm
- Has ***no established association*** with arterial aneurysms and dissections

Aneurysms and Dissections

- Occur most commonly in the aorta
- Can be present in other blood vessels
- Can be lethal if not detected or treated early
- Are attributed to genetic contributions and conventional risk factors
- Can be syndromic (e.g., Marfan Syndrome) or non-syndromic
- Can have lower thresholds for surgical repair if genetic cause is identified
- When genetic, can impact medical management and risk stratification of relatives
- Often have an ***unknown or unidentified genetic cause***

Methods

1. Clinical exome sequencing (ES) at Lab #1 (academic medical center)

A 52-year-old female with ***bilateral vertebral artery dissection, aortic aneurysm***, joint hypermobility, and fibromuscular dysplasia was found to carry two variants in *TNXB*.

- Likely pathogenic (LP) c.8791+1G>A and
- Variant of uncertain significance (VUS) c.12469+2T>C
- Segregation analysis confirmed orientation of variants is *in trans*

2. Retrospective review of clinical ES data from Lab #1

350 clinical exomes (ES) with the referral indication of aneurysm and/or dissection of various vessels were screened for variants in *TNXB.*

- 10/350 (2.8%) carried at least one variant in *TNXB*
  - **5/350 (1.4%) were found to carry two variants in *TNXB***
- All 5 had a personal history of aneurysm or dissection

Research limitation: lack of large dataset of matched controls

Proposed solution: data sharing partnership with commercial laboratory

3. Analysis of genetic testing options from other labs to identify replication dataset

Commercially available genetic tests were compared for inclusion or exclusion of *TNXB* in panels

- Search terms used: aneurysm, dissection, aortopathy, aortic aneurysm
- 22 labs offered panel testing for aneurysms and dissections
- Only ***3/22*** labs included *TNXB* in multigene panels (MGPT) for aneurysm/dissection
- Other labs included *TNXB* for connective tissue disease (CTD) but not aneurysms/dissections

4. Data analysis

Case-control analysis was conducted using a datasets from one commercial diagnostic laboratory, Lab #2. Analysis consisted of:

- MGPT and ES results
  - 1844 MGPT for clinical suspicion of thoracic aortic aneurysm with dissection (TAAD) from Lab#2 that analyzed *TNXB*
  - 27,068 exomes used as controls; 26,922 of these were provided by Lab#2
- Unclassified variants, VUSs, LP variants, and pathogenic variants in *TNXB*
- Fisher's Exact Test to analyze the burden of ≥2 *TNXB* variants between cases and controls

Results

Retrospective review

2.8% of cases from Lab #1 had at least one variant in *TNXB*.
1.43% of ES cases and 1.0% of MGPT cases carried 2 or more *TNXB* variants.
In contrast, the average allele frequency of single variants in *TNXB* is expected to be 0.29%.

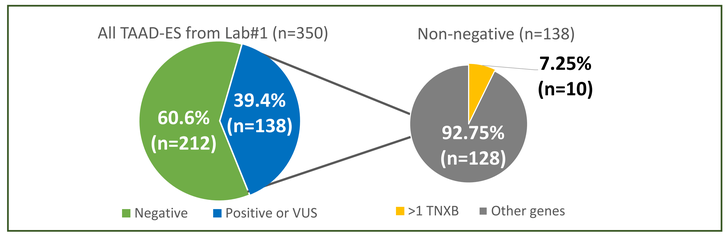


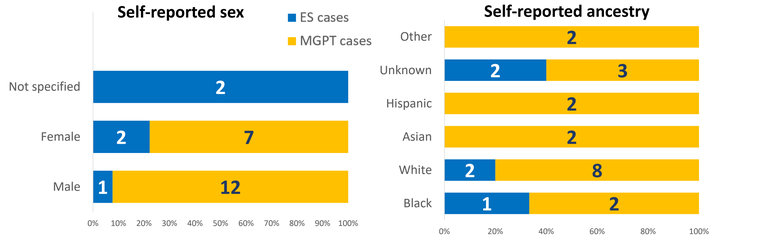
Figure 1
Results of retrospective review querying cases with *TNXB* variants from clinical exome data

Statistical analysis

There is a significant difference in the presence of >2 *TNXB* variants between cases & controls

Cases	Controls	Significant/ not significant	P value
Lab 1 (n=5/350)	n=89/27, 068	Significant	< .01
Lab 2 (n=19/1844)		Significant	

Demographic information



Phenotype information

All individuals from ES cases and most individuals from MGPT cases with ≥ 2 *TNXB* variants had a personal history of aneurysm or dissection (noted A/D below).

Figure 2A
Clinical information for ES cases

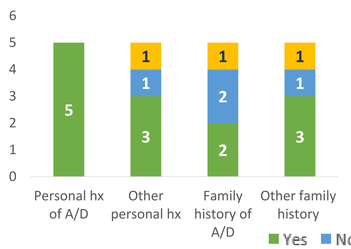
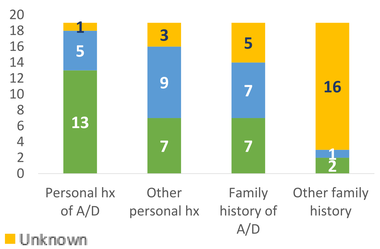
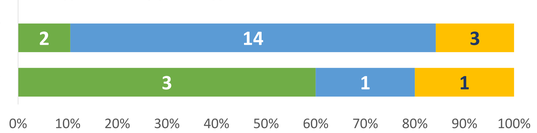


Figure 2B
Clinical information for MGPT cases



Unlike the known phenotype of clEDS, hypermobility was not present in a majority of MGPT cases

Figure 2C
Hypermobility phenotype for ES and MGPT cases

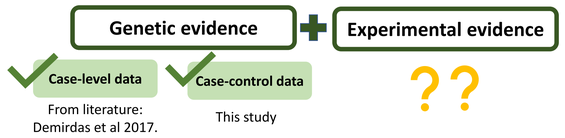


Implications

Our case-control data analysis provides the first statistical evidence of a vascular phenotype for *TNXB* using a large control dataset. There are several interrelated implications of this result, described below.

Gene curation

- *TNXB* is currently not validated as a disease gene for vascular aneurysms & dissections
- Our data provides case-control evidence for gene curation, supporting this association
- Additional experimental data will likely be required before universal inclusion on tests



Test selection

- Currently, *TNXB* is not universally included on tests for aneurysms & dissections
- Clinicians may be unaware of *TNXB* as a candidate gene for aneurysms & dissections
- Inconsistent inclusion of *TNXB* and limited testing of affected individuals stalls understanding of disease pathogenesis and genotype-phenotype correlation

Clinical implications

- Vascular aneurysms & dissections appear to be a rare feature of *TNXB,* but with potential significant clinical relevance for patients
- There may be an increased risk of vascular events regardless of cis/trans orientation
- Risk for A/D in 'carriers' in families with 2 or more variants is currently unknown and warrants additional exploration

CONCLUSION

Summary

- *TNXB* encodes tenascin-X, which plays an important role in maintaining the structural integrity of connective tissues.
- *TNXB* variants do not have an established association with vascular aneurysms or dissections
- After incorporating data from one commercial laboratory, we amassed significant evidence supporting the expansion of phenotype associated with *TNXB*
- This reinforces the value of transparent data sharing, within the confines of privacy regulations, between researchers, clinicians, and laboratories in the identification of candidate genes

Limitations

- Larger sample size is needed to evaluate genotype-phenotype correlation.
- *TNXB* is not routinely included in genetic testing for A/D, and this data represent patients from only 2 laboratories
- Broader testing is needed to determine a more accurate prevalence of *TNXB* variants and risk of A/D to patients

References

1. Alcaraz, L. B., Exposito, J. Y., Chuvin, N., Pommier, R. M., Cluzel, C., Martel, S., . . . Valcourt, U. (2014). Tenascin-X promotes epithelial-to-mesenchymal transition by activating latent TGF-beta. J Cell Biol, 205(3), 409-428. doi: 10.1083/jcb.201308031
2. Faggion Vinholo, T., Brownstein, A. J., Ziganshin, B. A., Zafar, M. A., Kuivaniemi, H., Body, S. C., . . . Elefteriades, J. A. (2019). Genes Associated with Thoracic Aortic Aneurysm and Dissection: 2019 Update and Clinical Implications. Aorta (Stamford), 7(4), 99-107. doi: 10.1055/s-0039-3400233
3. Demirdas S, Dulfer E, Robert L, Kempers M, van Beek D, Micha D, van Engelen BG, Hamel B, Schalkwijk J, Loeys B, Maugeri A, Voermans NC. Recognizing the tenascin-X deficient type of Ehlers-Danlos syndrome: a cross-sectional study in 17 patients. Clin Genet. 2017 Mar;91(3):411-425. doi: 10.1111/cge.12853. Epub 2016 Nov 4. PMID: 27582382.
4. Ostberg, N. P., Zafar, M. A., Ziganshin, B. A., & Elefteriades, J. A. (2020). The Genetics of Thoracic Aortic Aneurysms and Dissection: A Clinical Perspective. Biomolecules, 10(2). doi: 10.3390/biom10020182
5. Zweers, M. C., Bristow, J., Steijlen, P. M., Dean, W. B., Hamel, B. C., Otero, M., . . . Schalkwijk, J. (2003). Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet, 73(1), 214-217. doi: 10.1086/376564

COI: MT is a salaried employee of Ambry Genetics. AN and AM are employees of Yale University. The authors declare no conflicts of interest.