SUPPLEMENTAL MATERIAL

METHODS

Study cohort: Patients less than 18 years old diagnosed with (i) Williams-Beuren syndrome (WBS) based on clinical evaluation and/or cytogenetic confirmation of 7q11.23 deletion, or (ii) non-syndromic supravalvar aortic stenosis (SVAS) with/without those with elastin (ELN) variants were screened in a single center retrospective cohort study. Only patients with a confirmed cardiovascular lesion were included. Data were collected from patient medical records and included demographics, family history, genetic test results, age at cardiac diagnosis, cardiovascular lesion type, dates and types of catheter and/or surgical interventions and re-interventions, and history of hypertension (defined as need for anti-hypertensive medications for systemic hypertension during follow-up). The presence or absence of syndromic features was determined through clinical evaluation by a clinical geneticist and/or a cardiologist. The study was approved by the institutional Research Ethics Board and waiver of consent was obtained for retrospective analysis of clinical data. In the subset of patients that were enrolled in the Heart Centre Biobank Registry with collection of DNA and tissue samples for research, DNA was used to confirm genetic diagnosis on a research basis. Written informed consent for biobanking was obtained from the patient, parent or legal guardian and the protocol was approved by the institutional Research Ethics Board. In addition, arterial samples acquired at the time of surgical repair from consented patients were retrieved from the biobank or from leftover tissue in Surgical Pathology and evaluated histologically for structural abnormalities and elastin expression in the arterial wall.
**Genetic testing:** In patients consented to the biobank in whom clinical genetic testing reports were not available in our institutional medical records (e.g. those with genetic testing done at the referring site), banked DNA was used for research genetic testing. This included multiplex ligation-dependent probe amplification to confirm 7q11.23 deletion (The Centre for Applied Genomics, Hospital for Sick Children, Toronto, ON), and *ELN* sequencing in non-syndromic patients without a deletion (GeneDx, Gaithersburg, MD). Coding regions and splice junctions were amplified and capillary sequencing was performed with bidirectional sequence assembly, aligned to NCBI RefSeq transcripts and human genome build hg19. Variant pathogenicity was determined using American College of Medical Genetics criteria.¹

To map the variants, R (version 3.6.1) and the GenomeGraphs Bioconductor package (version 1.46.0) were used to generate a map of the *ELN* sequence. Gene and transcript information was retrieved from Ensembl GRCh37 Biomart, under the IDs ENSG00000049540 for the gene information and ENST00000252034 for the transcript information. Exon numbering and label positions were derived from RefSeq NM_000501.3, Swiss-Prot P15502.3, and RefSeq RNA NM_001278939.1. The latter was used for exon 22, which is not present in either of the other sequences.

**Histological analysis:** In patients enrolled in the biobank, aortic and/or pulmonary artery tissue was obtained at surgery and was snap frozen in liquid nitrogen and formalin fixed for tissue processing. Six patients with transposition of the great arteries
(TGA) were used as controls and findings were compared to 7 WBS patients and 1 non-syndromic SVAS patient harboring an *ELN* variant. Samples were fixed in buffered formalin, processed and embedded in paraffin. Sections were stained with Movat's pentachrome in the histology laboratory (Department of Pathology and Laboratory Medicine, Hospital for Sick Children, Toronto, ON). This stain highlights elastic fibers, collagen, ground substance, fibrin and muscle. Additional immunostaining was performed for calponin, a smooth muscle marker (clone CALP) and CD68, a macrophage marker (Clone PG-MI) (Dako, Mississauga, Ontario) (Supplemental Table 1). Stained sections were digitalized with a Pannoramic 250 Flash II slide scanner (3DHISTECH, Budapest, Hungary). The photographs were analyzed with automated image analysis software (Image J, National Institutes of Health, Bethesda, Maryland). Elastic fiber fragmentation was measured as the number of segmented or broken elastic fibers (stained in black) per field, and the area of calponin and CD68 staining (brown) were averaged in 3 fields per section.

**Statistical analysis:** Data were expressed as means and medians, standard deviations, and ranges for continuous variables. Demographic, clinical and intervention data were compared between WBS and non-syndromic SVAS patients using Chi-square test or Fisher exact test for categorical variables and Student T-test or Mann-Whitney U test for continuous variables. Kaplan-Meier survival analysis was used to determine freedom from systemic hypertension requiring anti-hypertensive treatment, surgical and catheter intervention-free survival and time to re-intervention. Cox proportional hazard model was used to compare risk of surgical or catheter intervention
between two groups adjusted for sex. For re-interventions and hypertension, log-rank test was used for comparison between the two groups. Intervention and re-intervention rates were also compared per 100 patient years using exact mid-P method. Differences in arterial wall architecture between WBS and TGA patients were compared using paired Student t test. P<0.05 was considered significant. All statistical analyses were performed using Stata version 16.
SUPPLEMENTAL REFERENCES

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the american college of medical genetics and genomics and the association for molecular pathology. *Genet. Med.* 2015;17:405-424.
## Supplemental Table 1: Antibody characteristics

| Antibody | Species     | Clone | Code number | Source | Titer       | Validation                                                                 |
|----------|-------------|-------|-------------|--------|-------------|-----------------------------------------------------------------------------|
| Calponin | Mouse       | CALP  | M3556       | Dako   | Ready to use| Optimized staining for sensitivity and specificity were performed using the Dako Omnis platform. |
|          | anti-human  |       |             | Omnis  |             | Ten positive and 10 negative cases were tested.                              |
|          |             |       |             |        |             | All method, results were assessed by the pathologist.                      |
|          |             |       |             |        |             | Once approved, the method was validated.                                   |
| CD68     | Mouse       | PG-M1 | GA613       | Dako   | Ready to use| Optimized staining for sensitivity and specificity were performed using the Dako Omnis platform. |
|          | anti-human  |       |             | Omnis  |             | Ten positive and 10 negative cases were tested.                            |
|          |             |       |             |        |             | All method, results were assessed by the pathologist.                      |
|          |             |       |             |        |             | Once approved, the method was validated.                                   |