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Vascular Biomechanical Properties in Mice with Smooth Muscle Specific Deletion of *Ndst1*

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Abstract

Heparan sulfate proteoglycans (HSPG) act as co-receptors for many chemokines and growth factors. The sulfation pattern of the heparan sulfate chains is a critical regulatory step affecting the binding of chemokines and growth factors. N-deacetylase-N-sulfotransferase1 (*Ndst1*) is one of the first enzymes to catalyze sulfation. Previously published work has shown that HSPGs alter tangent moduli and stiffness of tissues and cells. We hypothesized that loss of *Ndst1* in smooth muscle would lead to significant changes in heparan sulfate modification and the elastic properties of arteries. In line with this hypothesis, the axial tangent modulus was significantly decreased in aorta from mice lacking *Ndst1* in smooth muscle (SM22 α Cre⁺*Ndst1*^{-/-}, $p < 0.05$, $n = 5$). The decrease in axial tangent modulus was associated with a significant switch in myosin and actin types and isoforms expressed in aorta and isolated aortic vascular smooth muscle cells. In contrast, no changes were found in the compliance of smaller thoracodorsal arteries of SM22 α Cre⁺*Ndst1*^{-/-} mice. In summary, the major findings of this study were that targeted ablation of *Ndst1* in smooth muscle cells results in altered biomechanical properties of aorta and differential expression of myosin and actin types and isoforms.

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Keywords

Ndst1; Heparan Sulfate Proteoglycan; arterial compliance; tangent moduli; biomechanics

Introduction

Heparan sulfate proteoglycans (HSPGs) are highly abundant molecules on the cell membrane and in the extracellular matrix [1]. HSPGs are composed of polysaccharide chains attached to a core protein [2,3]. The polysaccharide chains are composed of alternating disaccharide units of N-acetylglucosamine and glucuronic acid residues [4]. Biosynthesis of HSPG polysaccharide chains involves a series of enzymatic reactions. Post translational modifications include N- and O-sulfations of the disaccharide subunits of the heparan sulfate side chains [5,6,7]. Sulfated domains specify the location of ligand binding sites. N-deacetylase-N-sulfotransferase1 (*Ndst1*), one out of the four *Ndst* isoforms, plays a dominant role in forming N-sulfated domains for ligand binding to the heparan sulfate side chains [8].

HSPG molecules have the ability to bind a variety of ligands via the sulfated domains, influencing the bioactivities of many substances such as lipids, chemokines and growth factors [5]. HSPGs have been implicated in several pathological conditions including atherosclerosis and restenosis [9] cardiovascular disease [10,11] and diabetes [12], brain cancer [13] Alzheimer's disease [14].

Arterial stiffness is a risk factor for cardiovascular disease [15,16]. Several reports in the literature demonstrate that HSPGs alter elasticity and stiffness in tissues and cells [17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38]. The objective of this study was to determine if the elastic properties of the vessel and smooth muscle cell may be mechanistically mediated through the modification of heparan sulfate chains which bind extracellular matrix and modulate expression of smooth muscle cell marker genes [39]. Thus determining if altering levels of N- and 2-O sulfation significantly modulates arterial stiffness is novel and may be important in predicting cardiovascular risk. Previous data from our laboratory has shown that level of *Ndst1* increases 40 fold following vascular injury in mice [40]. Others have shown that the expression of proteoglycans that modulate tissue stiffness is altered following vascular intervention or atherosclerosis [12,41,42,43,44,45,46].

Materials & Methods

Generation of *Ndst1* deficient mouse models

Ndst1^{flox/flox} mice (gift from Dr. JD. Esko) were mated with male SM22^{acre} mice (gift from Dr. M. Parmacek). F1 SM22^{acre}⁺*Ndst1*^{wt/flox} males were mated with *Ndst1*^{flox/flox} females to generate mice with smooth muscle specific deletion of *Ndst1* (SM22^{acre}⁺*Ndst1*^{-/-}). All the mice used for this study were of the C57BL6 strain. Studies were performed on 3-4 months old male mice. The genotype of wild type (WT) control mice was SM22^{acre}⁻*Ndst1*^{wt/wt}. Generation of SM22^{acre}⁺*Ndst1*^{-/-} has been described previously

[47]. Mice were maintained on normal diet and water ad libitum. Mice were euthanized according to our approved IACUC protocol with a compressed air carbon dioxide chamber.

Illumina MouseWG-6 v2 Expression BeadChip array

Total RNA from thoracic aorta pooled from two WT and two SM22 α cre⁺Ndst1^{-/-} male mice was extracted by Trizol. Following cleanup (RNeasy Mini-elute Cleanup Kit, Qiagen), 1-2 micrograms per sample of total RNA was submitted to the Biomedical Genomics Center for Illumina Direct Hybridization processing. Quality control was performed using the NanoDrop 8000 (Thermo Fisher Scientific, Waltham, MA, USA) and Caliper LabChip GX (Caliper Life Sciences, Hopkinton, MA, USA). Biotin-labeled cRNA was created using the Illumina TotalPrep RNA Amplification kit (Life Technologies, Carlsbad, CA, USA).

300ng total RNA was used in the first-strand reaction, creating single stranded cDNA. An *in vitro* transcription (IVT) reaction of the double stranded cDNA yielded amplified biotin-labeled antisense cRNA. Prior to hybridization cRNA concentration was determined using the NanoDrop 8000. 150 ng of biotinylated cRNA from both the WT (n=1) and SM22 α cre⁺Ndst1^{-/-} (n=1) was then hybridized in triplicate onto the Illumina MouseWG-6 v2 Expression BeadChip array (Illumina, San Diego, CA, USA) as instructed in Illumina's Whole-Genome Gene Expression Direct Hybridization Assay Guide. The BeadChip was then scanned by the Illumina iScan System, and the data package was assembled using Illumina GenomeStudio Data Analysis software. Annotations for Illumina probe sets were sourced from the University of Cambridge ReMOAT table version 1.0.0 [48]. Raw data files can be viewed at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44345>.

The canonical pathways and functional analyses were generated through the use of IPA (Ingenuity Systems, www.ingenuity.com). Data was statistically analyzed and transcripts with false discovery rate (FDR) meeting the filtering criteria (FDR < 0.05) was used as input to IPA. This is represented in Supplemental Table I. This table lists genes ranked according to q values in the SM22 α cre⁺Ndst1^{-/-} aorta. The q value represents the minimum FDR at which a test can be called significant. IPA searches known canonical pathways involving one or more members of the list and evaluates the probability of randomly assigning that number of genes to the pathway using the fisher exact test. A Benjamini-Hochberg correction for multiple testing is then applied. This generates the probability (p-value) of significance for that pathway. Table 4 lists 10 of the top pathways with their respective corrected p-values and gene members.

Isolation of vascular smooth muscle cells

Vascular smooth muscle cell from thoracic aorta of 3-4 months old male WT (n=5) and SM22 α cre⁺Ndst1^{-/-} (n=5) mice were isolated by enzyme digestion according to Ray et al, [49]. Thereafter, cells were maintained in DMEM supplemented with 10%FBS and 1% penicillin and streptomycin. Cells were used at passage 2 for gene expression analysis.

Real Time Quantitative PCR (RTQPCR)

RNA was extracted from primary isolated aortic vascular smooth muscle cells (n=5 per cohort) or aorta from WT and SM22 α cre⁺Ndst1^{-/-} mice by Trizol (Invitrogen). RNA from

thoracic aorta used for RTQPCR included n = 1 from the original beadchip and n=4 from new mice from each cohort. cDNA was synthesized (Invitrogen) and transcript abundance of target genes was assessed by RTQPCR by using Taqman primer probes as described previously [47,50]. Expression of targets was normalized to HPRT1, a housekeeper gene, and relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Values are expressed as mean $\pm$ SE.

Morphometry

From the aortic arch, the first 4 mm of the vessel was embedded in paraffin for morphometry (See Figure 1A). The sections were stained with hematoxylin and eosin. Images were taken using the Zeiss Axio Imager M1 Upright Microscope and all measurements were performed using Axiovision LE 4.7 software. At least 4 sections from each animal were measured. The following parameters were measured by a blinded observer:

- Vessel area = area delimited by external elastic lamina
- Luminal area = area delimited by intima
- Vessel diameter = diameter across the external elastic lamina
- Luminal diameter = diameter across the lumen delimited by intima.

From these measurements, vessel wall area was calculated as the difference between vessel area and luminal area. Vessel wall thickness was calculated as half the difference between vessel diameter and luminal diameter. Sections of vessels from both the cohorts were stained with Verhoeff-Van Gieson stain for visualization of elastin and with Masson's trichrome stain for visualization of collagen content, respectively.

Uniaxial Mechanical Testing-Aorta

Thoracic aorta sections (~6 mm in axial length, Figure 1A) were clamped longitudinally with a gauge length of 2 mm and subjected to uniaxial stretching on a mechanical test machine (Instron, Norwood, MA). Prior to stretching, a small pre-load of 0.005 N was applied to the vessels. Tests were performed under displacement control, stretching the vessels uniaxially at 1% per second for 450 seconds while recording tension via a force transducer. Stress-strain curves were generated from these measurements and tangent

modulus was calculated using the following formula: $E = \frac{\text{tensile stress}}{\text{tensile strain}} = \frac{\sigma}{\epsilon} = \frac{\frac{F}{A_0}}{\frac{\Delta L}{L_0}} = \frac{F L_0}{A_0 \Delta L}$

Where F is the force exerted under tension; A_0 is the original cross-sectional vessel wall area through which the force is applied (in this study, measured by morphometry: vessel wall area was calculated as the difference between vessel area and luminal area (Fig 1A)); L is the change in length; L_0 is the original gauge length (i.e., 2mm).

Compliance-TDA

Thoracodorsal arteries (TDA) free of surrounding tissue were placed in an arteriograph (Danish MyoTechnology, DMT), where they were cannulated at both ends with glass micropipettes and secured with 10-0 nylon monofilament suture as previously described

[51]. Arteries were perfused with Krebs-HEPES supplemented with 1% BSA and superfused with a calcium free Krebs-HEPES containing ethylenedis-(oxyethyleninitrolo) tetraacetic acid (EGTA, 2 mmol/L) and sodium nitroprussiate (10 μ mol/L). The arteriograph was placed on an Olympus IX-71 microscope, the TDAs were visualized with a 20 $\times$ objective and were subjected to a gradient of pressure from 10 to 140 mmHg with a 5 minute stabilization period for each pressure to measure the passive diameter using the Slidebook software. The intraluminal pressure was increased from 10 mmHg to 140 mmHg by steps of 20 mmHg and the lumen diameter was measured in μ m using the Slidebook software. The lumen area was calculated in μ m², for each pressure step, using the lumen diameter values according to the formula: lumen area = $\pi \times \text{lumen diameter}^2/4$. The compliance was measured in μ m²/mmHg using the formula: compliance = lumen area/ pressure in which lumen area is the change in lumen area induced by a change of intraluminal pressure (pressure).

Statistical Analysis

A Student's T-test was used to compare compliance and axial tangent moduli measurements between WT and SM22 α cre⁺Ndst1^{-/-}. $P < 0.05$ was considered significant. All values presented are mean $\pm$ SE.

RNA expression values from the Illumina MouseWG-6 v2 Expression BeadChip array was normalized using quantile normalization. Fold change between WT and SM22 α cre⁺Ndst1^{-/-} triplicates was calculated for each transcript, and the statistical significance was assessed using a two-sample t-test with unequal variance assumption (on log₂ scale). The FDRs were calculated with Benjamini and Hochberg's method [52]. Transcripts were discarded if expression of no more than one out of the three replicates were significantly above background (detection p-value < 0.01) for both WT and SM22 α cre⁺Ndst1^{-/-}.

Results

Morphometry

Figure 1A illustrates the region of aorta from 3-4 months old male SM22 α cre⁺Ndst1^{-/-} mice utilized for morphometric analysis. Both the aortic vessel and luminal area were significantly smaller in SM22 α cre⁺Ndst1^{-/-} mice in concordance with our previous findings [53]. There was no significant difference in aortic wall thickness (Figure 1B, Table 1).

Uniaxial mechanical testing - Aorta

Aorta segments were stretched uniaxially. This is in contrast to the methodology used for the TDAs. Axial measurements in the aorta have been used more often in the coming years to compare different strains and different pathological conditions [54,55,56]. 6 mm segments of thoracic aorta were stretched uniaxially. Figure 2A represents stress-strain curves of WT and SM22 α cre⁺Ndst1^{-/-} aorta. Tangent moduli of SM22 α cre⁺Ndst1^{-/-} (n=5) were significantly lower than those of WT (n=11) (Figure 2B). This indicates that the aortas from SM22 α cre⁺Ndst1^{-/-} mice showed altered tangent modulus in the axial direction than the WT mice at baseline. However, tangent moduli values in the toe-region (Figure 2A), low strain

portion of the stress-strain curves, were not significantly different between groups (WT 0.15 ± 0.02 , $n=11$ vs. SM22 α cre⁺Ndst1^{-/-} 0.13 ± 0.03 , $n=5$, $p=ns$).

Gene expression array and Staining

The decreased tangent moduli of the thoracic aorta from mice lacking the *Ndst1* enzyme in smooth muscle were associated with significant changes in overall gene expression. Table 2 lists genes whose expression differs in Ndst1 deficient mice ranked according to q values and with a cut off at a fold change of 2.0. Significant changes were noted in muscle isoforms and types expressed in smooth muscle including myosin light chain polypeptide 1, 4 and 7 (*Myh11*, 4 and 7) and heavy chain polypeptide 6 (*Myh6*), cardiac alpha actin (*Actc-1*), actinin 2 (*Actn2*), troponin C (*Tnntc1*) and Ankyrin repeat domain-1 (*Ankrd-1*). Genes regulating mitochondrial respiration including mitochondrial creatine kinase 2 (*Ckm-2*) and cytochrome oxidase subunit 6a2 (*Cox6a2*) were also significantly up-regulated in SM22 α cre⁺Ndst1^{-/-} aorta along with Sarcolipin (*Sln*), a regulator of the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA), cardiac LIM protein, (*Csrp-3*) and natriuretic peptide precursor type A (*Nppa*). A list of significantly increased transcripts confirmed by RTQPCR in aorta from SM22 α cre⁺Ndst1^{-/-} are listed in Table 3. Data was statistically analyzed and transcripts ranked according to the q value were used as input to IPA (Supplemental Table I). Analysis of pathways by IPA identified significantly over represented groups of genes that identified several pathways in *Ndst1* deficient mice (Table 4).

In order to separate out the expression of candidate genes that modulate contractility and elasticity in vascular smooth muscle cells from other cell types in the aorta, mRNA analysis was performed in isolated primary vascular smooth muscle cells and the whole aorta. There was no significant difference in the expression of collagen1 α 1 (*Col1a1*), collagen1 α 2 (*Col1a2*), smooth muscle myosin heavy chain (*Myh11*), calponin (*Cnn1*), caldesmon (*Cald1*), elastin (*Eln*), myocardin (*Myocd*), and smooth muscle α actin (*Acta2*) either in isolated vascular smooth muscle cells or aorta from both the cohort (Table 5).

To further follow up on elastin and collagen protein expression and localization, we stained sections with Verhoeff Van Gieson and Masson's trichrome. Elastin was not different between the cohorts. This is shown in representative figures in 3A. Collagen content in femoral arteries (we did not stain aortas) was not significantly different between the cohorts as assessed by Masson's trichrome staining as shown in representative Figures in 3B. Finally, a previous publication from our group noted significant decreases in both N- and 2-O sulfation state of heparan sulfate in vessels from the mice lacking the *Ndst1* enzyme in smooth muscle vs. WT controls [47].

Compliance -TDAs

Circumferential compliance can be measured using different stretching methods. In the present study, the method used for the TDA involved stretching by gradually increasing the intraluminal pressure. These experiments are done in calcium-free medium to avoid a myogenic response of the vessel in presence of calcium. We previously reported that blood pressure, as measured by telemetry in conscious mice, was not significantly different in the SM22 α cre⁺Ndst1^{-/-} mice compared to WT mice [53]. We assessed compliance

measurements in TDAs from SM22 α cre⁺Ndst1^{-/-} (n=6) and WT (n=8). No significant differences were reported at any luminal pressure (Figure 4A). The percent change in compliance in SM22 α cre⁺Ndst1^{-/-} was not different as compared to WT despite a significant decrease in luminal area in SM22 α cre⁺Ndst1^{-/-} ($p<0.05$, Figure 4B). Smaller luminal area of TDAs in SM22 α cre⁺Ndst1^{-/-} mice has been previously published by our group [53]. The change in luminal diameter in response to increases in luminal pressure is shown in Figure 4C. In addition, wall thickness of TDAs was not different between the cohorts (Table 6). Given there were no significant differences, we did not perform any gene expression analyses on the TDAs but instead focused our gene expression analyses on the aortas.

Discussion

The major findings of this study were that targeted ablation of *Ndst1* in smooth muscle results in a decreased axial tangent moduli of aortas in parallel with significant differences in the isoform expression of actin, myosin, and troponin as well as other smooth muscle marker genes.

In uniaxial tests, aortas from SM22 α cre⁺Ndst1^{-/-} demonstrated a decreased axial tangent moduli compared to WT aortas. To further define the potential mechanisms behind the altered morphometry and biomechanical properties, an unbiased transcript analysis was performed. Surprisingly, loss of *Ndst1* in smooth muscle did not induce changes in the expression of candidate genes involved in elasticity, particularly collagen and elastin (in whole aorta or isolated aortic vascular smooth muscle cells). In addition, neither elastin nor collagen content or localization differed between the cohorts.

Most of the medial extracellular matrix is made up of collagen, elastin, and proteoglycans. Proteoglycans have been shown to be important for the regulation of smooth muscle cell phenotype. Among the proteoglycans, heparin was shown to promote the maintenance of a contractile phenotype and slow down the proliferation of porcine and bovine smooth muscle cells [57,58]. Perlecan, another proteoglycan, was reported to inhibit smooth muscle cell proliferation through its heparan sulphate side chains by sequestering FGF2 [45]. Also, the expression of perlecan was negatively regulated by PDGF isoforms, which affected smooth muscle cells migration [59].

We identified novel changes in myosin and actin isoforms including *Myh11*, 4 and 7 and *Myh6*, *Actc-1*, *Actn2*, *Tnntc1*. *Actc1*, is primarily a cardiac specific muscle gene. However, expression has been seen in endothelial cells [60] and mesothelial cells, the precursor cells of vascular smooth muscle cells in visceral organs [61]. Several examples of *Actc1* expression in vascular smooth muscle are found in the gene expression omnibus. *Actc1* is up ~ 2 fold in vascular smooth muscle in mice lacking *Ndst1*. Previous work has shown that heparan sulfate does alter muscle development [62]. *Actn2*, is also found primarily in skeletal muscle and heart. However several examples of *Actc2* expression in smooth muscle are also found in gene expression omnibus. *Actn2* expression is up 3 fold in vascular smooth muscle in mice lacking *Ndst1*. Ankyrin repeat domain 1, *Ankrd1*, interacts with the intermediate filament desmin [63], and is up-regulated ~ 5 fold in mice lacking *Ndst1*. Desmin is known to influence compliance of airways (composed of vascular smooth muscle

cells) and carotid arteries [22,63]. Also, heparin treatment increased the desmin levels of spindle-shaped porcine smooth muscle cells and inhibited their proliferation [58]. Further studies will be needed to determine if an increase in *Ankrd1* mediates the altered tangent modulus in the axial direction.

Cytokine inducible SH2 containing protein, *Cish*, is a member of the SOCS family and a negative regulator of inflammatory signaling [64,65]. *Cish* has also been shown to negatively regulate JAK2/Stat signaling [66]. The role of *Cish* in the vessel wall has not been investigated, yet it is up-regulated ~4.8 fold in mice lacking *Ndst1*. Creatine kinase mitochondrial 2, *Ckmt2*, is critical to energy metabolism and is up-regulated ~3.9 fold in mice lacking *Ndst1*. A recent paper in mice lacking heparanase shows a significant increase in energy balance and metabolism [67]. Moreover, loss of heparan sulfate in *Drosophila* alters mitochondrial localization and this may be happening within vascular smooth muscle cells [68].

Cardiomyopathy-associated protein 5, *Cmya5*, or myospryn, is involved in protein kinase A signaling and vesicular trafficking [69]. Interestingly, *Cmya5* is also a binding partner of desmin [70]. This gene did not make the stringency cutoff of a false discovery rate cutoff of 0.05. Cysteine and glycine rich protein 3, *Csrp3*, is a member of the LIM domain proteins, and was cloned originally from denervated skeletal muscle. *Csrp3* is a mechanostretch sensing gene [71], plays an important role in vascular smooth muscle [72] and is up-regulated 3.9 fold in mice lacking *Ndst1*. Chemokine ligand 9, *Cxcl9*, has been shown to disrupt the endothelial barrier and induce chemotaxis [73]. *Cxcl9* is a T-cell chemoattractant for leukocytes and is up-regulated 1.8 fold in mice lacking *Ndst1*. How loss of N – and 2-O sulfation increase expression of *Cxcl9* is not clear.

One of the top pathways identified by IPA was “Cellular effects of the vasodilator sildenafil”. This is in direct association with the decreased axial tangent moduli recorded in the aorta. Cardiac muscle myosin, *Myh6* is one of the major building blocks of cardiac muscle. *Myh6* encodes the alpha heavy chain and is up-regulated ~ 7 fold in mice lacking *Ndst1*. In mice, adult cardiac myocytes primarily express *Myh6*. Examples in gene expression omnibus show expression of *Myh6* in human, rat and mouse vascular smooth muscle cells. Guanylate cyclase 1 alpha-3 (*Gucy1a3*) functions as the main receptor for nitric oxide and nitrovasodilator drugs and is up-regulated 1.2 fold in mice lacking *Ndst1*. It was recently identified in GWAS associated with coronary artery disease in Han Chinese [74], and blood pressure in 200,000 individuals of European descent [75]. Fully differentiated smooth muscle cells express alpha actin, non-muscle beta-actin, non-muscle gamma-actin and smooth muscle gamma actin. The role of beta-actin (*Actb*) is not clear and it is down-regulated by half in mice lacking *Ndst1* compared to WT controls. Atrial natriuretic factor (*Nppa*; upregulated > 3.6 fold in mice lacking *Ndst1* in smooth muscle) and phosphodiesterase 4 (*PDE4*; downregulated to ~ 0.7007) are acute vasodilating agents [76,77]. Phosphorylation of the myosin light chains correlates with shortening velocity of smooth muscle. The major myosin light chains in smooth muscle are myosin light chain 17 and 20. This pathway includes myosin light chain 1 and 7 (upregulated > 9 fold in mice lacking *Ndst1*) which are found primarily in skeletal and cardiac muscle, respectively. Thus again these findings see a significant shift in isoforms of myosin and the myosin light chains

in mice lacking N and 2-O sulfation that was associated with decreased axial tangent moduli in the aorta.

This second pathway was nitric oxide signaling in the cardiovascular system. This pathway included kinogen1, *Knlg1*, which stimulates production of nitric oxide. *Knlg1* is down-regulated in mice with loss of *Ndst1* in smooth muscle. Phospholamban, *Pln*, and the ryanodine 2 receptor are both expressed in cardiac muscle and are increased 1.8 and 1.7 fold respectively with loss of *Ndst1* in smooth muscle. The mechanism driving this increased expression of primarily cardiac calcium handling genes in smooth muscle lacking N and 2-O sulfation is not clear but has potential long term implications in aortic elasticity and stiffness.

We identified a significant decrease in the aortic axial tangent moduli in mice lacking *Ndst1*. Previous work from our group showed that these mice exhibit decreased N- and 2-O sulfation of heparan sulfate side chains in SM22 α cre⁺*Ndst1*^{-/-} mice [47]. Studies have also reported changes in HSPG biosynthesis are associated with altered wound repair, tissue/cellular elasticity and stiffness and contractility [20,23,47,60,64,78]. Deficiency of perlecan, a basement membrane proteoglycan, was reported to alter the elastic moduli of the pericellular matrix of chondrocytes [37] and muscle stiffness in patients with Schwartz-Jampel syndrome [36,78]. Syndecan-4 was reported to control actomyosin contractility through Rho kinase signaling in tissues and cells [34]. Similarly, Syndecan-3 was reported to coordinate myometrial contractility of smooth muscle in the uterine wall [19]. Accumulation of heparan sulfates in patients with mycopolysaccharidosis (MPS) leads to joint stiffness [21] and an increase in ascending aortic stiffness [32]. In addition, HSPGs are known to enhance elastin deposition and assembly and maintain elasticity in aging fibroblasts [28].

No significant difference in compliance of the smaller TDAs was identified between the cohorts. Thus, loss of *Ndst1* in smooth muscle does not appear to alter compliance in the TDAs. The compliance measurement made on the TDA's was performed differently compared to the axial measurement performed on the aorta. Thus the two measurements cannot be compared. Previous finding from our laboratory showed that baseline arterial systolic and diastolic blood pressure in conscious SM22 α cre⁺*Ndst1*^{-/-} was not different from controls [41]. Thus, no difference in blood pressure between the cohorts aligns with the lack of difference in compliance in these smaller vessels.

The potential limitations of this study include the following. We do not know the extent to which the bronchi or intestinal smooth muscle cells are altered in the SM22 α cre⁺*Ndst1*^{-/-}. This may have in an indirect fashion affected the size of the aorta and TDAs.

In conclusion, loss of N- and 2-O sulfation due to smooth muscle specific deletion of *Ndst1* resulted in a significant decrease in aorta size, and a decrease in aortic tangent moduli. These findings offer insights into the mechanisms through which loss of sulfation of HSPG molecules alters biomechanical properties of the aorta that are accompanied by novel changes in gene expression that are found in pathways that alter vasodilation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Esko JD, Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Ann Rev Biochem.* 2002; 71:435–71.10.1146/annurev.biochem.71.110601.135458 [PubMed: 12045103]
2. Kim SH, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *Journal of Endocrinology.* 2011; 209:139–151.10.1530/joe-10-0377 [PubMed: 21307119]
3. Kirkpatrick CA, Selleck SB. Heparan sulfate proteoglycans at a glance. *J Cell Sci.* 2007; 120:1829–1832.10.1242/jcs.03432 [PubMed: 17515480]
4. Lander AD, Selleck SB. The elusive functions of proteoglycans: in vivo veritas. *J Cell Biol.* 2000; 148:227–232.10.1083/jcb.148.2.227 [PubMed: 10648554]
5. Sarrazin S, Lamanna WC, Esko JD. Heparan Sulfate Proteoglycans. *Cold Spring Harbor Perspectives in Biology.* 2011; 310.1101/cshperspect.a004952
6. Selleck SB. Genetic analysis of functions for cell surface proteoglycans. *Matrix Biol.* 1998; 17:473–476.10.1016/S0945-053X(98)90094-4 [PubMed: 9881598]
7. Selleck SB. Proteoglycans and pattern formation: sugar biochemistry meets developmental genetics. *Trends Genet.* 2000; 16:206–12.10.1016/S0168-9525(00)01997-1 [PubMed: 10782114]
8. Sheng J, Liu R, Xu Y, Liu J. The Dominating Role of N-Deacetylase/N-Sulfotransferase 1 in Forming Domain Structures in Heparan Sulfate. *Journal of Biological Chemistry.* 2011; 286:19768–19776.10.1074/jbc.M111.224311 [PubMed: 21454625]
9. Wight TN, Merrilees MJ. Proteoglycans in Atherosclerosis and Restenosis. *Circulation Research.* 2004; 94:1158–1167.10.1161/01.res.0000126921.29919.51 [PubMed: 15142969]
10. Takahashi R, Negishi K, Watanabe A, Arai M, Naganuma F, Ohyama Y, Kurabayashi M. Serum syndecan-4 is a novel biomarker for patients with chronic heart failure. *Journal of Cardiology.* 2011; 57:325–332.10.1016/j.jjcc.2011.01.012 [PubMed: 21397460]
11. Vanhoutte D, Schellings MWM, Götte M, Swinnen M, Herias V, et al. Increased Expression of Syndecan-1 Protects Against Cardiac Dilatation and Dysfunction After Myocardial Infarction. *Circulation.* 2007; 115:475–482.10.1161/circulationaha.106.644609 [PubMed: 17242279]
12. Tannock L, King V. Proteoglycan mediated lipoprotein retention: A mechanism of diabetic atherosclerosis. *Reviews in Endocrine and Metabolic Disorders.* 2008; 9:289–300.10.1007/s11154-008-9078-0 [PubMed: 18584330]
13. Wade A, Robinson AE, Engler JR, Petritsch C, James CD, Phillips JJ. Proteoglycans and their roles in brain cancer. *FEBS Journal.* 2013; 280:2399–2417.10.1007/s11154-008-9078-0 [PubMed: 23281850]
14. Cotman SL, Halfter W, Cole GJ. Agrin Binds to β -Amyloid ($A\beta$), Accelerates $A\beta$ Fibril Formation, and Is Localized to $A\beta$ Deposits in Alzheimer's Disease Brain. *Molecular and Cellular Neuroscience.* 2000; 15:183–198.10.1006/mcne.1999.0816 [PubMed: 10673326]
15. Chirinos J. Arterial Stiffness Basic Concepts and Measurement Techniques. *Journal of Cardiovascular Translational Research.* 2012; 5:243–255.10.1007/s12265-012-9359-6 [PubMed: 22447229]
16. Duprez DA, Cohn JN. Arterial stiffness as a risk factor for coronary atherosclerosis. *Curr Atheroscler Rep.* 2007; 9:139–144.10.1007/s11883-007-0010-y [PubMed: 17877923]
17. Balasubramani M, Schreiber EM, Candiello J, Balasubramani GK, Kurtz J, Halfter W. Molecular interactions in the retinal basement membrane system: A proteomic approach. *Matrix Biology.* 2010; 29:471–483.10.1016/j.matbio.2010.04.002 [PubMed: 20403434]
18. Buczek-Thomas JA, Chu CL, Rich CB, Stone PJ, Foster JA, Nugent MA. Heparan sulfate depletion within pulmonary fibroblasts: Implications for elastogenesis and repair. *Journal of Cellular Physiology.* 2002; 192:294–303.10.1002/jcp.10135 [PubMed: 12124775]

19. Cluff A, Bystrom B, Klimaviciute A, Dahlqvist C, Cebers G, Malmstrom A, Ekman-Ordeberg G. Prolonged labour associated with lower expression of syndecan 3 and connexin 43 in human uterine tissue. *Reproductive Biology and Endocrinology*. 2006; 4:24.10.1186/1477-7827-4-24 [PubMed: 16674815]
20. Elenius V, Götte M, Reizes O, Elenius K, Bernfield M. Inhibition by the Soluble Syndecan-1 Ectodomains Delays Wound Repair in Mice Overexpressing Syndecan-1. *Journal of Biological Chemistry*. 2004; 279:41928–41935.10.1074/jbc.M404506200 [PubMed: 15220342]
21. Feldhammer M, Durand S, Mrázová L, Boucher RM, Laframboise R, et al. Sanfilippo syndrome type C: mutation spectrum in the heparan sulfate acetyl-CoA: a-glucosaminide N-acetyltransferase (HGSNAT) gene. *Human Mutation*. 2009; 30:918–925.10.1002/humu.20986 [PubMed: 19479962]
22. Gambillara V, Thacher T, Silacci P, Stergiopoulos N. Effects of Reduced Cyclic Stretch on Vascular Smooth Muscle Cell Function of Pig Carotids Perfused Ex Vivo. *American Journal of Hypertension*. 2008; 21:425–431.10.1038/ajh.2007.72 [PubMed: 18219296]
23. Gheduzzi D, Guerra D, Bochicchio B, Pepe A, Tamburro AM, et al. Heparan sulphate interacts with tropoelastin, with some tropoelastin peptides and is present in human dermis elastic fibers. *Matrix Biology*. 2005; 24:15–25.10.1016/j.matbio.2004.12.001 [PubMed: 15748998]
24. Götte M, Spillmann D, Yip GW, Versteeg E, Echtermeyer FG, van Kuppevelt TH, Kiesel L. Changes in heparan sulfate are associated with delayed wound repair, altered cell migration, adhesion and contractility in the galactosyltransferase I (β 4GalT-7) deficient form of Ehlers-Danlos syndrome. *Human Molecular Genetics*. 2008; 17:996–1009.10.1093/hmg/ddm372 [PubMed: 18158310]
25. Hocking DC, Kowalski K. A cryptic fragment from fibronectin's III1 module localizes to lipid rafts and stimulates cell growth and contractility. *The Journal of Cell Biology*. 2002; 158:175–184.10.1083/jcb.200112031 [PubMed: 12105189]
26. Hultgårdh-Nilsson A, Durbeek M. Role of the extracellular matrix and its receptors in smooth muscle cell function: implications in vascular development and disease. *Current Opinion in Lipidology*. 2007; 18:540–545.10.1097/MOL.0b013e3282ef77e9 [PubMed: 17885425]
27. Huveneers S, Truong H, Fässler R, Sonnenberg A, Danen EHJ. Binding of soluble fibronectin to integrin α 5 β 1 - link to focal adhesion redistribution and contractile shape. *Journal of Cell Science*. 2008; 121:2452–2462.10.1242/jcs.033001 [PubMed: 18611961]
28. Isnard N, Fodil-Bourahla I, Robert AM, Robert L. Pharmacology of skin aging. Stimulation of glycosaminoglycan biosynthesis by L-fucose and fucose-rich polysaccharides, effect of in vitro aging of fibroblasts. *Biomedicine & Pharmacotherapy*. 2004; 58:202–204.10.1016/j.biopha.2003.07.002 [PubMed: 15082343]
29. Jimenez-Vergara AC, Munoz-Pinto DJ, Becerra-Bayona S, Wang B, Iacob A, Hahn MS. Influence of glycosaminoglycan identity on vocal fold fibroblast behavior. *Acta Biomaterialia*. 2011; 7:3964–3972.10.1016/j.actbio.2011.06.034 [PubMed: 21740987]
30. Marucha J, Tytki-Szymahska A, Jakóbkiewicz-Banecka J, Piotrowska E, Kloska A, Czartoryska B, W grzyn G. Improvement in the range of joint motion in seven patients with mucopolysaccharidosis type II during experimental gene expression-targeted isoflavone therapy (GET IT). *American Journal of Medical Genetics Part A*. 2011; 155:2257–2262.10.1002/ajmg.a.34146 [PubMed: 21834048]
31. Negrini D, Tenstad O, Passi A, Wiig H. Differential degradation of matrix proteoglycans and edema development in rabbit lung. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2006; 290:L470–L477.10.1152/ajplung.00310.2005 [PubMed: 16214813]
32. Nemes A, Timmermans RM, Wilson JHP, Soliman OI, Krenning B, Cate FJ, Geleijnse ML. The mild form of mucopolysaccharidosis type I (Scheie syndrome) is associated with increased ascending aortic stiffness. *Heart and Vessels*. 2008; 23:108–111.10.1007/s00380-007-1013-x [PubMed: 18389335]
33. O'Callaghan R, Job KM, Dull RO, Hlady V. Stiffness and heterogeneity of the pulmonary endothelial glycocalyx measured by atomic force microscopy. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2011; 301:L353–L360.10.1152/ajplung.00342.2010 [PubMed: 21705487]

34. Okina E, Manon-Jensen T, Whiteford JR, Couchman JR. Syndecan proteoglycan contributions to cytoskeletal organization and contractility. *Scandinavian Journal of Medicine & Science in Sports*. 2009; 19:479–489.10.1111/j.1600-0838.2009.00941.x [PubMed: 19538537]
35. Schwinn MK, Gonzalez JM Jr, Gabelt BAT, Sheibani N, Kaufman PL, Peters DM. Heparin II domain of fibronectin mediates contractility through an $\alpha 4\beta 1$ co-signaling pathway. *Experimental Cell Research*. 2010; 316:1500–1512.10.1016/j.yexcr.2010.03.010 [PubMed: 20302860]
36. Stum M, Girard E, Bangratz M, Bernard V, Herbin M, et al. Evidence of a dosage effect and a physiological endplate acetylcholinesterase deficiency in the first mouse models mimicking Schwartz–Jampel syndrome neuromyotonia. *Human Molecular Genetics*. 2008; 17:3166–3179.10.1093/hmg/ddn213 [PubMed: 18647752]
37. Wilusz RE, DeFrate LE, Guilak F. A biomechanical role for perlecan in the pericellular matrix of articular cartilage. *Matrix Biology*. 2012; 31:320–327.10.1016/j.matbio.2012.05.002 [PubMed: 22659389]
38. Yang JJ, Chen YM, Kurokawa T, Gong JP, Onodera S, Onodera S, Yasuda K. Gene expression, glycocalyx assay, and surface properties of human endothelial cells cultured on hydrogel matrix with sulfonic moiety: Effect of elasticity of hydrogel. *Journal of Biomedical Materials Research Part A*. 2010; 95A:531–542.10.1002/jbm.a.32875 [PubMed: 20681030]
39. Shi ZD, Abraham G, Tarbell JM. Shear Stress Modulation of Smooth Muscle Cell Marker Genes in 2-D and 3-D Depends on Mechanotransduction by Heparan Sulfate Proteoglycans and ERK1/2. *PLoS ONE*. 2010; 5:e12196.10.1371/journal.pone.0012196 [PubMed: 20808940]
40. Adhikari N, Rusch M, Li Q, Selleck SB, Hall JL. Significant alterations in heparan sulfate biosynthesis in response to vascular injury. *Circulation*. 2004; 11:III–335.
41. Bingley JA, Hayward IP, Campbell JH, Campbell GR. Arterial heparan sulfate proteoglycans inhibit vascular smooth muscle cell proliferation and phenotype change in vitro and neointimal formation in vivo. *J Vasc Surg*. 1998; 28:308–318.10.1016/S0741-5214(98)70167-3 [PubMed: 9719326]
42. Segev A, Nili N, Strauss BH. The role of perlecan in arterial injury and angiogenesis. *Cardiovasc Res*. 2004; 63:603–610.10.1016/j.cardiores.2004.03.028 [PubMed: 15306215]
43. Theocharis AD, Theocharis DA, De Luca G, Hjerpe A, Karamanos NK. Compositional and structural alterations of chondroitin and dermatan sulfates during the progression of atherosclerosis and aneurysmal dilatation of the human abdominal aorta. *Biochimie*. 2002; 84:667–674.10.1016/S0300-9084(02)01428-1 [PubMed: 12453639]
44. Tovar AMF, Teixeira LAC, Marinho ACO, Pinho DA, Silva LF, Mourão PAS. The dermatan sulfate-dependent anticoagulant pathway is mostly preserved in aneurysm and in severe atherosclerotic lesions while the heparan sulfate pathway is disrupted. *Clinica Chimica Acta*. 2011; 412:906–913.10.1016/j.cca.2011.01.016
45. Tran PK, Tran-Lundmark K, Soininen R, Tryggvason K, Thyberg J, Hedin U. Increased intimal hyperplasia and smooth muscle cell proliferation in transgenic mice with heparan sulfate-deficient perlecan. *Circ Res*. 2004; 94:550–8.10.1161/01.RES.0000117772.86853.34 [PubMed: 14739157]
46. Tran-Lundmark K, Tran PK, Paulsson-Berne G, Friden V, Soininen R, et al. Heparan Sulfate in Perlecan Promotes Mouse Atherosclerosis: Roles in Lipid Permeability, Lipid Retention, and Smooth Muscle Cell Proliferation. *Circ Res*. 2008; 103:43–52.10.1161/CIRCRESAHA.108.172833 [PubMed: 18596265]
47. Adhikari N, Basi DL, Townsend D, Rusch M, Mariash A, Mullegama S, Watson A, Larson J, et al. Heparan sulfate Ndst1 regulates vascular smooth muscle cell proliferation, vessel size and vascular remodeling. *Journal of Molecular and Cellular Cardiology*. 2010; 49:287–293.10.1016/j.yjmcc.2010.02.022 [PubMed: 20206635]
48. Barbosa-Morais NL, Dunning MJ, Samarajiwa SA, Darot JFJ, Ritchie ME, Lynch AG, Tavaré S. A re-annotation pipeline for Illumina BeadArrays: improving the interpretation of gene expression data. *Nucleic Acids Res*. 2010; 38:e17.10.1093/nar/gkp942 [PubMed: 19923232]
49. Ray JL, Leach R, Herbert JM, Benson M. Isolation of vascular smooth muscle cells from a single murine aorta. *Methods Cell Sci*. 2001; 23:185–188.10.1023/A:1016357510143 [PubMed: 12486328]

50. Adhikari N, Basi DL, Carlson M, Mariash A, Hong Z, et al. Increase in GLUT1 in Smooth Muscle Alters Vascular Contractility and Increases Inflammation in Response to Vascular Injury Arteriosclerosis Thrombosis and Vascular Biology. 2011; 31:86–94.10.1161/atvbaha.110.215004
51. Billaud M, Lohman AW, Straub AC, Parpaite T, Johnstone SR, Isakson BE. Characterization of the Thoracodorsal Artery: Morphology and Reactivity. Microcirculation. 2012; 19:360–372.10.1111/j.1549-8719.2012.00172.x [PubMed: 22335567]
52. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological). 1995; 57:289–300.10.2307/2346101
53. Montaniel, Kim; Ramil, C.; Billuad, M.; Graham, C.; Kim, SK.; Carlson, M.; Zeng, W.; Zeng, O.; Pan, W., et al. Smooth Muscle Specific Deletion of Ndst1 Leads to Decreased Vessel Luminal Area and No Change in Blood Pressure in Conscious Mice. Journal of Cardiovascular Translational Research. 2012; 5:274–279.10.1007/s12265-012-9369-4 [PubMed: 22555965]
54. Agianniotis A, Stergiopoulos N. Wall properties of the apolipoprotein E-deficient mouse aorta. Atherosclerosis. 2012; 223:314–320.10.1016/j.atherosclerosis.2012.06.014 [PubMed: 22770991]
55. Eberth JF, Popovic N, Gresham VC, Wilson E, Humphrey JD. Time course of carotid artery growth and remodeling in response to altered pulsatility. American Journal of Physiology - Heart and Circulatory Physiology. 2010; 299:H1875–H1883.10.1152/ajpheart.00872.2009 [PubMed: 20852047]
56. Hayenga HN, Trache A, Trzeciakowski J, Humphrey JD. Regional Atherosclerotic Plaque Properties in ApoE^{-/-} Mice Quantified by Atomic Force, Immunofluorescence and Light Microscopy. Journal of Vascular Research. 2011; 48:495–504.10.1159/000329586 [PubMed: 21832839]
57. Frid MG, Aldashev AA, Dempsey EC, Stenmark KR. Smooth Muscle Cells Isolated From Discrete Compartments of the Mature Vascular Media Exhibit Unique Phenotypes and Distinct Growth Capabilities. Circulation Research. 1997; 81:940–952.10.1161/01.res.81.6.940 [PubMed: 9400374]
58. Hao H, Ropraz P, Verin V, Camenzind E, Geinoz A, Pepper MS, Gabbiani G, Bochaton-Piallat ML. Heterogeneity of Smooth Muscle Cell Populations Cultured From Pig Coronary Artery. Arteriosclerosis Thrombosis and Vascular Biology. 2002; 22:1093–1099.10.1161/01.atv.0000022407.91111.e4
59. Koyama N, Kinsella M, Wight TN, Hedin U, Clowes AW. Heparan Sulfate Proteoglycans Mediate a Potent Inhibitory Signal for Migration of Vascular Smooth Muscle. Cells Circulation Research. 1998; 83:305–313.10.1161/01.res.83.3.305 [PubMed: 9710123]
60. Godman CA, Chheda KP, Hightower LE, Perdrizet G, Shin DG, Giardina C. Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells. Cell Stress and Chaperones. 2010; 15:431–442.10.1007/s12192-009-0159-0 [PubMed: 19949909]
61. Lachaud CC, Pezzolla D, Domínguez-Rodríguez A, Smani T, Soria B, Hmadcha A. Functional Vascular Smooth Muscle-like Cells Derived from Adult Mouse Uterine Mesothelial Cells. PLoS ONE. 2013; 8:e55181.10.1371/journal.pone.0055181 [PubMed: 23405120]
62. Zielinska M, Sawosz E, Grodzik M, Wierzbicki M, Gromadka M, et al. Effect of heparan sulfate and gold nanoparticles on muscle development during embryogenesis. International Journal of Nanomedicine. 2011; 6:3163–3172.10.2147/Ijn.S26070 [PubMed: 22238506]
63. Mohamed JS, Boriek AM. Loss of desmin triggers mechanosensitivity and up-regulation of Ankrd1 expression through Akt-NF- κ B signaling pathway in smooth muscle cells. The FASEB Journal. 2012; 26:757–765.10.1096/fj.10-160291 [PubMed: 22085644]
64. Hu G, Zhou R, Liu J, Gong AY, Eischeid AN, Dittman JW, Chen XM. MicroRNA-98 and let-7 Confer Cholangiocyte Expression of Cytokine-Inducible Src Homology 2-Containing Protein in Response to Microbial Challenge. The Journal of Immunology. 2009; 183:1617–1624.10.4049/jimmunol.0804362 [PubMed: 19592657]
65. Khor CC, Vannberg FO, Chapman SJ, Guo H, Wong SH, et al. CISH and Susceptibility to Infectious Diseases. New England Journal of Medicine. 2010; 362:2092–2101.10.1056/NEJMoa0905606 [PubMed: 20484391]

66. Ram PA, Waxman DJ. SOCS/CIS Protein Inhibition of Growth Hormone-stimulated STAT5 Signaling by Multiple Mechanisms. *Journal of Biological Chemistry*. 1999; 274:35553–35561. [PubMed: 10585430]
67. Karlsson-Lindahl L, Schmidt L, Haage D, Hansson C, Taube M, et al. Heparanase Affects Food Intake and Regulates Energy Balance in Mice. *PLoS ONE*. 2012; 7:e34313.10.1074/jbc.274.50.35553 [PubMed: 22479599]
68. Ren Y, Kirkpatrick CA, Rawson JM, Sun M, Selleck SB. Cell Type-Specific Requirements for Heparan Sulfate Biosynthesis at the Drosophila Neuromuscular Junction: Effects on Synapse Function, Membrane Trafficking and Mitochondrial Localization. *The Journal of Neuroscience*. 2009; 29:8539–8550.10.1523/jneurosci.5587-08.2009 [PubMed: 19571145]
69. Sarparanta J. Biology of myospryn: what's known? *Journal of Muscle Research and Cell Motility*. 2008; 29:177–180.10.1007/s10974-008-9165-6 [PubMed: 19140017]
70. Kouloumenta A, Mavroidis M, Capetanaki Y. Proper Perinuclear Localization of the TRIM-like Protein Myospryn Requires Its Binding Partner Desmin. *Journal of Biological Chemistry*. 2007; 282:35211–35221.10.1074/jbc.M704733200 [PubMed: 17872945]
71. Knöll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, et al. The Cardiac Mechanical Stretch Sensor Machinery Involves a Z Disc Complex that Is Defective in a Subset of Human Dilated Cardiomyopathy. *Cell*. 2002; 111:943–955.10.1016/S0092-8674(02)01226-6 [PubMed: 12507422]
72. Wang X, Li Q, Adhikari N, Hall JL. A role for muscle LIM protein (MLP) in vascular remodeling. *J Mol Cell Cardiol*. 2006; 40:503–509.10.1016/j.yjmcc.2006.01.005 [PubMed: 16519896]
73. Amatschek, S.; Lucas, R.; Eger, A.; Pflueger, M.; Hundsberger, H., et al. *Br J Cancer*. Cancer Research UK; 2011. CXCL9 induces chemotaxis, chemorepulsion and endothelial barrier disruption through CXCR3-mediated activation of melanoma cells; p. 469-479.
74. Lu X, Wang L, Chen S, He L, Yang X, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat Genet*. 2012; 44:890–894.10.1038/ng.2337 [PubMed: 22751097]
75. The International Consortium for Blood Pressure Genome-Wide Association Studies. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011; 478:103–109.10.1038/nature10405 [PubMed: 21909115]
76. Schudt, C.; Hatzelmann, A.; Beume, R.; Tenor, H. Phosphodiesterase Inhibitors: History of Pharmacology. In: Francis, SH.; Conti, M.; Houslay, MD., editors. *Phosphodiesterases as Drug Targets*. Springer Berlin Heidelberg; 2011. p. 1-46.
77. Withrington PG, Dhume VG, Croxton R, Gerbes AL. The actions of human atrial natriuretic factor on hepatic arterial and portal vascular beds of the anaesthetized dog. *British Journal of Pharmacology*. 1990; 99:810–814.10.1111/j.1476-5381.1990.tb13011.x [PubMed: 2141797]
78. Echaniz-Laguna A, Rene F, Marcel C, Bangratz M, Fontaine B, Loeffler JP, Nicole S. Electrophysiological studies in a mouse model of Schwartz–Jampel syndrome demonstrate muscle fiber hyperactivity of peripheral nerve origin. *Muscle & Nerve*. 2009; 40:55–61.10.1111/j.1476-5381.1990.tb13011.x [PubMed: 19367640]

Figure 1.

(A) Schematic identifying anatomical regions of mouse aorta used for morphometry and axial elastic tangent measurements. (B) Representative micrographs of hematoxylin and eosin stained cross sections of aorta from WT and SM22αcre⁺Ndst1^{-/-} mice (Magnification at 10×). The vessel and lumen area are significantly smaller in SM22αcre⁺Ndst1^{-/-} mice. Wall thickness was not different between the cohorts (see table 1). IEL- Internal elastic lamina; EEL-External elastic lamina.

Figure 2.

(A) Representative stress-strain curves of aorta from WT and SM22 α cre+Ndst1 $^{-/-}$ mice. (B) Tangent moduli of WT (n=11) and SM22 α cre+Ndst1 $^{-/-}$ (n=5) aorta. SM22 α cre+Ndst1 $^{-/-}$ aorta has a significantly lower ($P=0.0002$) tangent modulus compared to WT. 6 mm longitudinal segments of thoracic aorta were clamped, given a preload of 0.005 N and then stretched uniaxially. Tangent moduli values in the toe-region, low strain portion of the stress-strain curves, were not significantly different between groups

Figure 3.

Representative micrographs of (A) Verhoeff Van Gieson stained cross sections of aorta from WT and SM22 α cre $^{+}$ Ndst1 $^{-/-}$ mice (Magnification at 10 $\times$). Elastin content or localization was not different between the cohorts. (B) Masson's trichrome stained cross sections of femoral arteries from WT and SM22 α cre $^{+}$ Ndst1 $^{-/-}$ mice (Magnification at 20 $\times$). Collagen content or localization was not different between the cohorts. IEL- Internal elastic lamina; EEL- External elastic lamina.

Figure 4.

(A) Compliance measurements between WT (n=6) and SM22 α cre+Ndst1 $^{-/-}$ (n=8) TDA at different luminal pressures. Measurements were made after a stepwise increase in luminal pressure. No significant differences in compliance were observed between WT and SM22 α cre+Ndst1 $^{-/-}$ TDAs at all pressures measured. (B) No significant difference in % change in compliance was observed. (C) Luminal diameter of SM22 α cre+Ndst1 $^{-/-}$ was significantly smaller at all pressures measured (*p=0.001).

Table 1

SM22 α cre⁺Ndst1^{-/-} (n= 8) exhibit a significantly smaller aortic vessel area, luminal area and intimal area compared to WT (n= 6). Smaller luminal area in SM22 α cre⁺Ndst1^{-/-} mice has been previously reported by our laboratory [53]. There were no significant differences in wall thickness between the two cohorts.

Measurements on aorta were made on a minimum of 4-5 hematoxylin and eosin stained sections per animal.

All values are mean $\pm$ SE.

Measurements	WT (n=6)	SM22 α cre ⁺ Ndst1 ^{-/-} (n=8)	P Value
Vessel Area (μm^2)	273,088 $\pm$ 16,575	210,578 $\pm$ 12,686*	0.02
Luminal Area (μm^2)	166,024 $\pm$ 18,774	126,026 $\pm$ 13,279*	0.04
Intimal area (μm^2)	2201 $\pm$ 226	1216 $\pm$ 231*	0.02
Wall Thickness (μm)	64.1 $\pm$ 2.7	58.8 $\pm$ 1.2	0.19

Table 2

List of significantly up-regulated and down-regulated genes with a fold change cut off above 2.0 and with q value <0.05 in SM22acre+Ndst1^{-/-} aorta as assessed by Illumina MouseWG-6 v2 Expression BeadChip array. Fold change between WT and SM22acre+Ndst1^{-/-} triplicates was calculated for each transcript, and the p value was assessed using a two-sample t-test with unequal variance assumption (on log2 scale). The false discovery rates (FDR) were calculated with Benjamini and Hochberg's method [9] and are represented as “q-values”.

Up-regulated Genes					
Probe_id	Gene_symbol	Gene_description	Fold Change	P value	q value
ILMN_2629581	Cox6a2	Cytochrome c oxidase, subunit VI a, polypeptide 2	9.2	3.90E-08	0.002
ILMN_2598916	Actc1	Actin, alpha, cardiac muscle 1	9.3	1.32E-07	0.003
ILMN_2610744	My14	Myosin, light polypeptide 4	8.0	1.41E-07	0.003
ILMN_2718330	Cish	Cytokine inducible SH2-containing protein	4.9	2.83E-07	0.003
ILMN_2918875	Slh	Sarcolipin	10.6	3.27E-07	0.003
ILMN_2503052	Tnni1	Troponin C, cardiac/slow skeletal	5.4	4.12E-07	0.004
ILMN_2749152	Segb1a1	Secretoglobin, family 1A, member 1 (uterogloblin)	3.5	5.74E-07	0.004
ILMN_3161547	Nppa	Natriuretic peptide precursor type A	3.6	6.49E-07	0.004
ILMN_2768252	My17	Myosin, light polypeptide 7, regulatory	9.8	7.81E-07	0.004
ILMN_1213817	Mup3	Major urinary protein 3	2.5	7.85E-07	0.004
ILMN_3128792	Ttn	Titin	3.5	8.47E-07	0.004
ILMN_1239742	Atp2a2	ATPase, Ca+++ transporting, cardiac muscle, slow twitch 2	2.6	8.80E-07	0.004
ILMN_1234662	Mb	Myoglobin	5.0	9.18E-07	0.004
ILMN_1216602	My11	Myosin, light polypeptide 1	3.5	1.43E-06	0.005
ILMN_2789650	Csrp3	Cysteine and glycine-rich protein 3	6.6	1.61E-06	0.005
ILMN_2817864	Ckm2	Creatine kinase, mitochondrial 2	3.9	1.75E-06	0.005
ILMN_1236304	Hamp	Hepcidin antimicrobial peptide	3.2	1.84E-06	0.005
ILMN_2689426	Mybphl	Myosin binding protein H-like	5.6	1.89E-06	0.005
ILMN_2666990	Eef1a2	Eukaryotic translation elongation factor 1 alpha 2	2.1	3.44E-06	0.008
ILMN_2950286	Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)	5.4	4.06E-06	0.008
ILMN_2954987	Mb	Myoglobin	6.3	5.11E-06	0.01
ILMN_2788836	Myh6	Myosin, heavy polypeptide 6, cardiac muscle, alpha	7.1	6.01E-06	0.011
ILMN_2764727	Actn2	Actinin alpha 2	3.1	6.29E-06	0.011

Up-regulated Genes					
Probe_id	Gene_symbol	Gene_description	Fold Change	P value	q value
ILMN_2710905	S100a8	S100 calcium binding protein A8 (calgranulin A)	2.2	8.36E-06	0.011
ILMN_2753867	Segb3a2	Secretoglobulin, family 3A, member 2	2.0	1.00E-05	0.012
ILMN_1234857	Myoz2	Myozenin 2	2.4	1.11E-05	0.012
ILMN_1259206	Hrc	Histidine rich calcium binding protein	2.1	1.38E-05	0.013
ILMN_2416670	Ttn	Titin	3.7	1.43E-05	0.013
ILMN_2625451	Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)	3.1	1.50E-05	0.013
ILMN_2593554	Igtp	Interferon gamma induced GTPase	2.2	2.56E-05	0.017
ILMN_2698052	Ckmt2	Creatine kinase, mitochondrial 2	3.7	3.68E-05	0.02
ILMN_1245425	Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	2.5	3.87E-05	0.02
ILMN_2588815	Pgam2	Phosphoglycerate mutase 2	2.7	4.05E-05	0.021
ILMN_2900484	Trim72	Tripartite motif-containing 72	2.8	4.18E-05	0.021
ILMN_2789651	Csrp3	Cysteine and glycine-rich protein 3	2.1	5.07E-05	0.023
ILMN_2971559	Eef1a2	Eukaryotic translation elongation factor 1 alpha 2	2.9	5.19E-05	0.024
ILMN_2742068	Csrp3	Cysteine and glycine-rich protein 3	3.9	6.52E-05	0.025
ILMN_2757569	Eno3	Enolase 3, beta muscle	2.1	7.21E-05	0.027
ILMN_1230991	Atp5g1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	2.1	1.12E-04	0.032
ILMN_2534635	abParts	Parts of antibodies, mostly variable regions.	2.0	1.21E-04	0.034
ILMN_2632262	abParts	Parts of antibodies, mostly variable regions.	2.1	1.49E-04	0.037
ILMN_3112219	Mif1	Myeloid leukemia factor 1	2.0	2.15E-04	0.044
Down-regulated genes					
Probe_id	Gene_symbol	Gene_description	Fold Change	P value	q value
ILMN_2616226	Dbp	D site albumin promoter binding protein	-3.2	2.60E-06	0.006
ILMN_1246800	Serpina3k	Serine (or cysteine) peptidase inhibitor, clade A, member3K	-2.8	7.39E-06	0.011
ILMN_2718431	Itih4	Inter alpha-trypsin inhibitor, heavy chain 4	-2.8	1.12E-05	0.012
ILMN_2668510	S67972	Haptoglobin	-2.6	1.71E-05	0.015
ILMN_1213609	Txnip	Thioredoxin interacting protein	-2.0	1.73E-05	0.015
ILMN_2712075	Lcn2	Lipocalin 2	-2.3	1.79E-05	0.015
ILMN_2668509	S67972	Haptoglobin	-2.6	2.50E-05	0.017

Up-regulated Genes					
Probe_id	Gene_symbol	Gene_description	Fold Change	P value	q value
ILMN_2627022	Itih4	Inter alpha-trypsin inhibitor, heavy chain 4	-2.5	5.71E-05	0.024
ILMN_2744890	Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma	-2.1	5.88E-05	0.024
ILMN_2944824	S67972	Haptoglobin	-2.2	1.49E-04	0.037
ILMN_2797276	Itih4	Inter alpha-trypsin inhibitor, heavy chain 4	-2.6	2.52E-04	0.046

Table 3

Confirmation of genes among the top up-regulated genes selected from the Illumina BeadChip array. Fold change in expression in SM22^{acre}+Ndst1^{-/-} (n=5) aorta relative to WT (n=5) was performed by RTQPCR. Values were normalized to HPRT1 expression and expressed as mean $\pm$ SE. Statistical significance was performed with the Student's T-test.

Probe ID	Target	Gene Symbol	RTQPCR		Array	
			Aorta	P value	Fold Change	q value
ILMN_2598916 ILMN_2767216	α actin cardiac muscle	<i>Actc-1</i>	86.6 $\pm$ 34.8	0.03	9.3 1.9	0.003 0.025
ILMN_2764727 ILMN_2797061	Actinin α 2	<i>Actn-2</i>	11.9 $\pm$ 3.9	0.02	2.5 3.1	0.05 0.011
ILMN_2950286 ILMN_2625451	Ankyrin repeat domain 1	<i>Ankrd-1</i>	7.8 $\pm$ 2.8	0.03	5.4 3.1	0.008 0.013
ILMN_2718330	Cytokine inducible SH2 containing protein	<i>Cish</i>	3.6 $\pm$ 0.8	0.04	4.9	0.003
ILMN_2817864 ILMN_2698052	Creatine kinase mitochondrial 2	<i>Ckmt-2</i>	333.5 $\pm$ 112.1	0.01	3.9 3.7	0.005 0.02
ILMN_2706640 ILMN_2677339	Cardiomyopathy associated 5	<i>Cmya-5</i>	3.3 $\pm$ 1.1	0.04	1.3 1.9	0.088 0.031
ILMN_2742068 ILMN_2789651 ILMN_2789650	Cysteine and glycine rich protein 3	<i>Csrp-3</i>	174.5 $\pm$ 68.1	0.02	3.9 3.9 6.6	0.025 0.023 0.005
ILMN_1215862	Chemokine ligand 9	<i>Cxcl9</i>	2.4 $\pm$ 0.2	0.005	1.8	0.032

Table 4

List of 10 of the top pathways with their respective corrected p-values and gene members. The canonical pathways and functional analyses were generated through IPA (Ingenuity Systems, www.ingenuity.com). Data was statistically analyzed and transcripts with false discovery rate (FDR) for differential expression meeting our filtering criteria (FDR < 0.05) was used as input to IPA (Supplemental table I). Given a ranked list of genes and significance values (p-value, q-value or fold change, IPA searches known canonical pathways involving one or more members of the list and evaluates the probability of randomly assigning that number of genes to the pathway using the fisher exact test. A Benjamini-Hochberg correction for multiple testing is then applied. This generates the probability of significance for that pathway which is displayed in the table as p-value.

Ingenuity Canonical Pathways	p-value	Molecules
Cellular Effects of Sildenafil (Viagra)	0.008	MYH6,GUCY1A3,ACTB,MYL4,NPPA,PDE4D,ACTC1,MYL1,MYL7
Nitric Oxide Signaling in the Cardiovascular System	0.012	KNG1,PLN,GUCY1A3,PIK3R1,RYR2,VEGFC,ATP2A2
Actin Cytoskeleton Signaling	0.014	KNG1,MYH6,F2R,ACTN2,PIK3R1,ACTB,MYL4,ACTC1,TTN,MYL1,MYL7
Regulation of Actin-based Motility by Rho	0.015	ACTB,RHOT2,MYL4,ACTC1,MYL1,PI4KA,MYL7
ILK Signaling	0.015	MYH6,ACTN2,PIK3R1,ACTB,RHOT2,MYL4,VEGFC,ACTC1,MYL1,MYL7
RhoA Signaling	0.018	SEPT8,ACTB,MYL4,ACTC1,TTN,MYL1,PI4KA,MYL7
Calcium Signaling	0.049	MYH6,TNNC1,RYR2,MYL4,ACTC1,ATP2A2,MYL1,MYL7
Mitochondrial Dysfunction	0.055	COX17,RHOT2,NDUFA12,UQCRC1,SNCA,ATP5F1,COX6A2
Hepatic Fibrosis/Hepatic Stellate Cell Activation	0.059	MYH6,CXCL9,TIMP1,MYL4,VEGFC,MYL1,MYL7
Epithelial Adherens Junction Signaling	0.065	MYH6,ACTN2,ACTB,MYL4,ACTC1,MYL1,MYL7

Table 5

Confirmation of genes known to play a role in vascular elasticity and contractility selected from the Illumina BeadChip array. Fold change in expression of targets in vascular smooth muscle cells and aorta from SM22accre^{+/Ndst1}^{-/-} (n=3-5) was assessed by RTQPCR and the array.

Probe ID	Target	Gene symbol	RTQPCR			Array	
			VSMC	Aorta	P value	Aorta	q value
ILMN_2693895 ILMN_2710354 ILMN_2923445 ILMN_2710353 ILMN_1237242	Smooth muscle α actin	<i>Acta2</i>	1.2 $\pm$ 0.1	1.1 $\pm$ 0.3	P=ns	-1.1 1.1 -1.1 1.1 -1.2	0.29 0.34 0.49 0.42 0.34
ILMN_2687872	Collagen 1 α 1	<i>Col1a1</i>	1.5 $\pm$ 0.2	1.1 $\pm$ 0.4	P=ns	1.0	0.82
ILMN_1253806	Collagen 1 α 2	<i>Col1a2</i>	1.4 $\pm$ 0.2	1.2 $\pm$ 0.5	P=ns	1.3	0.15
ILMN_1221148	Calponin	<i>Cnn1</i>	0.9 $\pm$ 0.1	1.2 $\pm$ 0.5	P=ns	-1.0	0.69
ILMN_2697304	Elastin	<i>Eln</i>	Not tested	1.1 $\pm$ 0.4	P=ns	-1.1	0.22
ILMN_2626391 ILMN_1218766 ILMN_2435360 ILMN_3151642 ILMN_3072880	Myocardin	<i>Myocd</i>	1.3 $\pm$ 0.5	Not tested	P=ns	1.1 1.1 1.2 1.2 1.0	0.15 0.36 0.07 0.39 1
ILMN_1243652	Transgelin	<i>Tagln</i>	1.6 $\pm$ 0.2	Not tested	P=ns	1.1	0.62
ILMN_2622217	Smooth muscle Myosin Heavy chain	<i>Myh11</i>	1.6 $\pm$ 0.2	1.0 $\pm$ 0.1	P=ns	-1.1	0.36
ILMN_1254073 ILMN_2804261 ILMN_1232081	Caldesmon	<i>Cald1</i>	0.7 $\pm$ 0.2	1.1 $\pm$ 0.3	P=ns	1.1 1.1 1.1	0.55 0.26 0.65
ILMN_2997494	Lysyl oxidase	<i>Lox</i>	Not tested	1.1 $\pm$ 0.3	P=ns	1.2	0.22

Table 6

TDA area measurements were done at 10 mmHg transluminal pressure during the compliance study. Luminal area is significantly smaller in SM22 α cre+Ndst1^{-/-} mice as previously reported [53]. All values are mean $\pm$ SE.

Measurements	TDA		P value
	WT (n=8)	SM22 α cre+Ndst1 ^{-/-} (n=6)	
Vessel area (μm^2)	51128 $\pm$ 2558	38754 $\pm$ 2555*	0.001
Lumen area (μm^2)	16094 $\pm$ 795	11057 $\pm$ 356*	0.001
Vessel wall Thickness (μm)	55.7 $\pm$ 2.2	51.5 $\pm$ 3.9	0.371