BIOMECHANICAL PROPERTIES OF THE SKIN IN ARTERIAL TORTUOSITY SYNDROME

by

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ABSTRACT

Arterial tortuosity syndrome (ATS) is a rare, autosomal recessively inherited connective tissue disorder caused by biallelic loss of function mutations in *SLC2A10*. While *SLC2A10* and its product, a class III facilitative glucose transporter (GLUT10), have been implicated in ATS, the exact disease mechanism has not been fully elucidated. ATS is characterized by systemic medium and large artery lengthening and subsequent tortuosity, which significantly increases affected individual's risk of ischemic and hemorrhagic events as well as aortic dilation and dissection. Histologically, fibroblasts and vascular tissue of affected individuals have demonstrated a dysregulation of the extracellular matrix proteins, particularly reduced and fragmented elastin fibers. While most individuals with ATS possess cutaneous findings characteristic of connective tissue disorders, ranging from soft, hyperextensible skin to visibly lax skin in redundant folds, no study has quantified differences in the biomechanical properties of the skin between individuals with ATS and healthy individuals. In this study, rapid, non-invasive, *in vivo* cutaneous measurements were executed using the DermaLab® Combo SkinLab to determine the functional consequences on the skin of systemic ATS in a cohort of 8 individuals, compared to 28 of their unaffected relatives. Our affected population demonstrated significantly reduced elastic modulus (E) and viscoelastic modulus (VE) when compared to unaffected individuals. Affected individuals also exhibited increased skin retraction time. This research significantly impacts public health by

contributing to rare disease research, specifically by further characterizing the natural history of ATS and aiding in diagnosing the condition in the general population.

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PREFACE

I would like to thank my advisor Zsolt Urban and my committee members Andrea Durst, Ryan Minster, and Suneeta Madan-Khetarpal for all of their assistance not only with this project, but throughout my classes and clinical rotations as well. I would also like to thank Robin Grubs for being so essential to my development as a genetic counselor. Finally, the support from the arterial tortuosity syndrome community, especially the participants of this study and Andrea Taylor, the founder and president of A Twist of Fate, has been invaluable and inspiring. Thank you.

1.0 INTRODUCTION

Arterial tortuosity syndrome (ATS) is a rare connective tissue disorder with approximately 100 cases reported in the medical literature.[1] ATS is caused by biallelic loss of function mutations in the *SLC2A10* gene, which encodes a III facilitative glucose transporter, GLUT10.[2] The primary feature of ATS is widespread medium and large artery tortuosity, which predisposes affected individuals to aortic aneurysm and dissection as well as other ischemic events.[3] Systemic tortuosity is diagnosed with vascular imaging, most frequently magnetic resonance angiogram (MRA) but echocardiogram, angiography, or computed tomography angiography may also be used.[4] Variable systemic involvement has been noted in affected individuals, including additional cardiovascular anomalies, characteristic craniofacial features, as well as other generalized connective tissue disorder findings like soft, hyperextensible skin, hernias, multiple skeletal findings, and ocular issues.[5]

Numerous studies have demonstrated dysregulation of the extracellular matrix in patients with ATS[1,2,6], however a single pathological mechanism has not been elucidated. Histologically, the fibroblasts and vascular tissue in individuals with ATS have reduced external elastic lamina, fragmented internal elastic lamina, and increased collagen deposition.[1,7] Most individuals with ATS possess cutaneous findings, the severity of which range from thin, hyperextensible skin with a velvety texture to significantly loose and lax skin.[1] Despite these findings, the biomechanical properties of the skin have not been measured in individuals with ATS. Previous studies have

characterized significant differences in quantifiable skin properties in other connective tissue disorders, like Williams syndrome,[8] the cutis laxa family of connective tissue disorders,[9] and Ehlers-Danlos syndrome.[10] In this study, we sought to determine the functional consequences of systemic connective tissue disease in individuals affected with arterial tortuosity syndrome by measuring physical properties of the skin, including elasticity, viscoelasticity, epidermal hydration, and dermal thickness. Unaffected individuals were used as healthy controls.

We hypothesized that individuals with ATS would have reduced skin elasticity, viscoelasticity, and dermal thickness as well as increased skin retraction time, epidermal hydration, and collagen intensity compared to healthy controls. To test these hypotheses, we performed *in vivo* measurements using a DermaLab® Combo SkinLab instrument on individuals affected with ATS.

2.0 LITERATURE REVIEW

2.1 ARTERIAL TORTUOSITY SYNDROME

Arterial tortuosity syndrome (ATS) is a rare, autosomal recessively inherited connective tissue disorder that was first described in 1967 by Ertugrul who noted widespread arterial tortuosity in a ten-year-old female patient and presumed this finding was due to a yet-unnamed congenital elastin defect.[7] ATS is characterized by an increased risk of aortic aneurysm and dissection secondary to this severe systemic arterial tortuosity.[11] Arterial tortuosity syndrome is caused by biallelic loss of function mutations in the *SLC2A10* gene, which encodes a class III facilitative glucose transporter, GLUT10.[2] Couke and colleagues elucidated that mutations in *SLC2A10* are the responsible for ATS through homozygosity mapping of consanguineous families affected by ATS in 2003.[12] Histopathologic studies of affected arterial tissue have revealed a reduced external elastic lamina and a fragmented internal elastic lamina.[1,3,7] These studies began as early as the first description of ATS by Ertugrul, who noted reduced and fragmented elastin in an arterial sample from the first defined case.[7] Most recently, Beyens and colleagues noted disorganization and fragmentation of elastin in pulmonary artery biopsies of three affected individuals and an aortic biopsy from one affected individual examined. These biopsies, when stained with Picrosirius polarizaition staining demonstrated "increased and disorganized collagen deposition compared with the control samples."[1]

While the exact prevalence of ATS is unknown, it is widely accepted to fit the definition of a rare disease in the United States with around 100 affected individuals reported in the literature.[1] ATS is believed to occur in less than one in one million live births.[11] This may be an

underestimate due to high clinical variability, underdiagnosis, and misdiagnosis as other related connective tissue disorders, including Loeys-Dietz syndrome (LDS) and cutis laxa (CL).[5] For example, LDS patients may frequently present with arterial tortuosity, although this tortuosity and aneurysm formation is recognized to be less severe than in ATS.[13] Additionally, autosomal recessive forms of CL caused by mutations in the *LTBP4* gene and the *FBLN5* gene similarly cause generalized connective tissue disorder findings as well as pulmonary artery stenosis.[14] However, CL does not cause systemic arterial tortuosity.

ATS has proven to be a difficult disorder to research, not only due to its rarity in the population, but also due to its lack of an ideal model organism. While GLUT10 knockout mice have been bred to model arterial tortuosity, they exhibit a less severe or even entire lack of cardiovascular phenotype compared to humans affected with ATS.[15] A *slc2a10* knockout zebrafish has proven to be a superior phenocopy to human ATS, with embryos possessing a wavy notochord and cardiovascular anomalies.[16]

2.1.1 CARDIOVASCULAR CHARACTERISTICS

ATS is named for the abnormal lengthening of the large and medium-sized arteries, including the aorta and distributing arteries, which causes the vessels to convolute with bends and twists. ATS is specifically characterized by this distinct tortuosity in two or more vessels, as tortuosity of a single vessel is more common.[7] While any vessel can be affected, the head, neck, and pulmonary arteries are most commonly affected.[1] Figure 1 demonstrates this tortuosity captured through vascular imagining in the aorta and aortic side branches, the thoracic and abdominal aortas, and the intracranial arteries from left to right (Figure 1). Tortuosity increases shear stress on the vessel walls and inhibits normal blood flow.[17] Therefore, arterial tortuosity predisposes affected

individuals to aortic and arterial aneurysms, which may result in cerebrovascular accident or aortic dissection, significant causes of morbidity and mortality in individuals with ATS.[18,19] Several older patients and patients with a less severe diverse clinical presentation have been documented.[5] Affected individuals may also suffer from isolated or widespread vessel stenosis.[20] Pulmonary artery stenosis often presents with pulmonary hypertension. Venous tortuosity has also been observed in some patients.[21] While not fully elucidated, an alteration in the transforming growth factor-ß (TGFß) signaling pathway has been implicated in the mechanism of aberrant cardiovascular formation and functioning, akin to the pathophysiology of other connective tissue disorders including LDS and CL.[16]

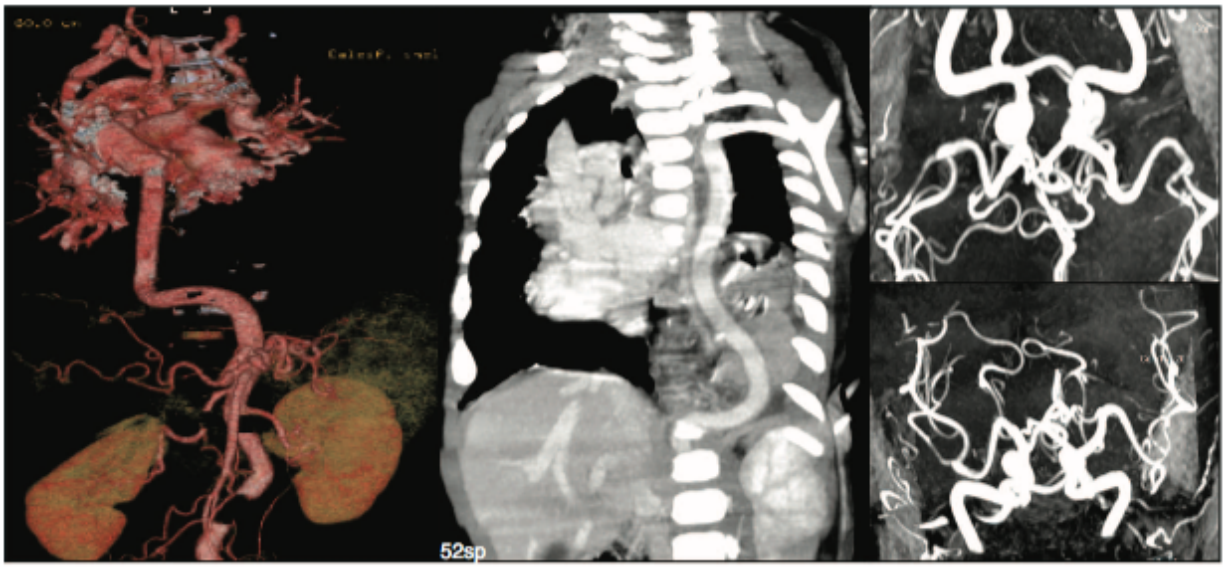

Figure 1. Vascular imaging of individuals affected with ATS

2.1.2 EXTRACARDIOVASCULAR CLINICAL CHARACTERISTICS

While extracardiovascular features have been noted in multiple individuals with ATS, no single extracardiovascular clinical characteristic is a pathognomic sign of the condition.

2.1.2.1 CRANIOFACIAL

Individuals with ATS may possess characteristic facial features, including an elongated face with midface retrusion and micrognathia.[20] A high/arched palate is another common, although not universal, craniofacial feature among affected individuals.[11] Hypertelorism, downslanting palpebral fissures, convex nasal ridge, and dental crowding have also been reported in individuals with ATS.[1] These craniofacial findings are not typically associated with significant complications, although small jaw and high palate can contribute to feeding difficulties in infancy, which may progress to failure to thrive if not managed appropriately.[11]

2.1.2.2 SKELETAL

Other skeletal features that typically manifest in connective tissue disorders may also be present in individuals with ATS. For example, affected individuals may exhibit scoliosis, pectus excavatum or pectus carinatum, joint laxity, joint contractures, arachnodactyly, and/or camptodactyly.[22]

2.1.2.3 OCULAR

Ocular findings of other connective tissue disorders are also present in ATS, particularly myopia and keratoconus.[23] Less frequently, keratoglobus has been noted in affected individuals.[1]

2.1.2.4 CUTANEOUS

Beyond skeletal findings, individuals with ATS often exhibit other classic characteristics of connective tissue disorders, including soft, hyperextensible skin, hernias (including inguinal and abdominal wall hernias as well as sliding hiatal or diaphragmatic hernias), hypotonia, and pelvic

organ prolapse.[24] Cutaneous findings in ATS are variable, ranging from velvety, hyperextensible skin characteristic of connective tissue disorders to noticeably loose and lax skin.[1,5]

2.1.2.5 RESPIRATORY

Infant respiratory distress syndrome, in which a neonate's lungs are underdeveloped leading to respiratory distress, has frequently been observed in the newborn period.[25] This initial respiratory issue resolves with the proper treatment and has not been shown to have lasting impact on lung function.[1]

2.1.3 DIAGNOSIS, PROGNOSIS, AND MANAGEMENT

Around half of individuals with ATS are diagnosed following a cardiovascular finding, ranging from a murmur heard on routine exam to spontaneous aortic dissection.[1] Onset is congenital, although the age of diagnosis may vary based on the severity of the clinical characteristics. Diagnostic criteria do not exist for ATS, as the systemic tortuosity is apparent on vascular imaging including magnetic resonance angiography (MRA), computed tomography (CT), echocardiogram, or angiography.[4] Individuals with widespread tortuosity on imaging typically undergo sequencing and deletion/duplication analysis of *SLC2A10* to molecularly confirm the diagnosis.[5]

While the prognosis of ATS has long been considered to be poor with only 40% surviving past five years of life,[22] the identification of the *SLC2A10* gene and increased utilization of expanded genetic testing has informed more recent reports suggesting that ATS has a more variable expression than previously believed.[5] Therefore, affected individuals have been diagnosed later in life into the second and third decades.[1]

There is no cure for arterial tortuosity syndrome. Affected individuals require regular evaluations to determine what interventions are necessary to their management. These evaluations include echocardiograms, vascular imaging, ophthalmic examinations, and evaluation of the palate.[26] If individuals present with symptoms, lung function imaging and testing as well as skeletal imaging may be warranted.[11] Several medications may be utilized to reduce stress on the arterial walls, including beta-adrenergic blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor I antagonists.[11] Arterial aneurysms and stenoses, including pulmonary artery stenosis, frequently require surgical intervention, either surgery, catherization, or a transcatheter-surgical procedure.[27,28] Potential skeletal issues are treated accordingly by an orthopedist and may include orthotics and potentially surgical intervention of the spine.[11] Similarly, eye manifestations are treated by an ophthalmologist and respiratory symptoms by a pulmonologist.

2.1.4 A TWIST OF FATE–ATS

A Twist of Fate-ATS (ATOF) is an international patient group for ATS. ATOF was established by a family that has been significantly impacted by ATS and has been a source of support for other families. ATOF has also grown to hold an annual multidisciplinary clinic and educational seminar with specialists from multiple fields, including genetics, cardiology, and ophthalmology. Today, the mission of ATOF is "to find a cure for arterial tortuosity syndrome, by supporting research, education, awareness, and families."[29]

2.1.5 GENE

ATS is caused by biallelic, pathogenic loss of function variants in the *SLC2A10* gene, located on the short arm of chromosome 20. *SLC2A10* is comprised of five exons and encodes the class three facilitative glucose transporter 10 (GLUT10), a member of the solute carrier family 2. The role of *SLC2A10* mutations in ATS was initially discovered in 2003 through homozygosity mapping of consanguineous ATS families. After identifying a 4.1 Mb region on chromosome 20q13.1, gene sequencing revealed homozygous loss of function mutations in the *SLC2A10* gene in all the studied families.[12] Three families in this research were all from the same region in Morocco, leading researchers to believe that there may be a founder mutation causing disease in this population. There are around nine recurrent mutations observed in affected individuals, notably a single nucleotide substitution of a cytidine to a thymidine at position 394 of the *SLC2A10* transcript (c.394C>T), which results in an arginine to tryptophan amino acid substitution in the protein (p.R132W). Some of these recurrent mutations exhibit founder affects ascertained through haplotype analysis.[21] While several different types of mutations have been observed including deletions and nonsense mutations, all causative mutations are loss of function mutations.[5] s

2.1.6 GENE PRODUCT

ATS is among the few connective tissue disorders where the responsible gene encodes a transport protein. GLUT10 is a facilitative glucose transporter that consists of 541 residues and contains twelve hydrophobic transmembrane domains.[16] Facilitative transporters, or uniporters, aid in the movement of impermeable solutes through the cellular membrane down a concentration gradient. GLUT10 structurally diverges from other facilitative glucose transporters with a longer exofacial

loop between the ninth and tenth transmembrane domains. GLUT10 also lacks both the N-terminal dileucine signal that other class III members have and the PESPR motif in loop six that all other GLUTs possess.[30] These differences have led researchers to suggest that GLUT10 may perform additional roles beyond the typical scope of other members of facilitative glucose transporters. GLUT10 has shown expression in multiple organs including the heart, liver, pancreas, lung, brain, skeletal muscle, and placenta.[31] In more specific ATS studies, GLUT10 expression has been identified in the aortic smooth muscle cells and adipocytes.[32] Previously, *SLC2A10* was identified as potential candidate gene for noninsulin-dependent diabetes mellitus through genome wide association studies due to GLUT10's role as a glucose transporter.[33] However, several studies have shown that mutations in *SLC2A10* do not result in a higher incidence of diabetes mellitus.[5,34]

2.1.7 PATHOPHYSIOLOGY

The solute carrier group of transport proteins typically localize to the cell membrane and many of their solutes have been identified; however, the exact cellular location and substrates of GLUT10 have only been partially elucidated, making the pathophysiology of ATS difficult to definitively ascertain.[35] GLUT10 deficiency has been associated with increased transforming growth factor ß (TGFß) signaling in the arterial wall, which has also been noted in Loeys-Dietz syndrome (LDS). This observation has been implicated in the phenotypic similarities between ATS and LDS.[2] Several studies have further explored these unknown factors in an attempt to determine the mechanism of ATS, detailed below.

2.1.7.1 TRANSFORMING GROWTH FACTOR ß SIGNALING

The TGFß signaling pathway is essential for embryonic development and a multitude of cellular processes including growth, differentiation, and apoptosis.[36] Importantly, TGFß has also been shown to be an essential inhibitor of angiogenesis and enhancer of blood vessel maturation.[37] The pathway consists of a superfamily of ligands, including bone morphogenetic proteins, growth and differentiation factors, anti-mullerian hormone, activin, nodal, and TGFß, binding to a superfamily of five receptors, specifically serine/threonine receptor kinases.[38] The activated receptors then phosphorylate receptor-regulated SMADs which bind to SMAD4, a common signaling transducer. The subsequent complexes act as transcription factors in the nucleus, regulating gene expression. Increased TGFß signaling has been implicated in the vascular pathogenesis of LDS, which shares clinical features with ATS, namely systemic arterial tortuosity and a high risk for aortic aneurysm and dissection.[39] However, gene expression and molecular rescue studies in a zebrafish model of ATS indicated decreased TGFß signaling through embryonic development.[16] Thus, increased TGFß signaling in postnatal vessels may represent a compensatory rather than pathogenic process.

2.1.7.2 GLUCOSE-DEPENDENT GENE EXPRESSION

Facilitative glucose transporters (GLUTs) are so named as glucose has been implicated as their primary substrate. Mutations in other *SLC* genes, which encode for other GLUTs, have been implicated in health conditions caused by dysregulation of glucose metabolism. For example, biallelic mutations in *SLC2A2* which encode for glucose transporter 2 (GLUT2) are responsible for Fanconi-Bickel syndrome, a glycogen storage disorder.[40] In ATS, researchers have observed decreased glucose levels within fibroblasts. This decreased intracellular glucose level is believed to affect gene expression of decorin, which directly influences TGFß signaling. Decorin is a proteoglycan which associates with connective tissue fibers that inhibit TGFß signaling. This

hypothesis is supported by evidence of increased phosphorylation and nuclear translocation of Smad2 and connective tissue growth factor as well as decreased decorin expression in vascular smooth muscle cells. Of note, the same changes between individuals with ATS and healthy controls was not noted in fibroblast cells.[2] This data is correlative, and it remains unclear whether the observed molecular changes contribute to the disease, are casually unrelated markers of the disease, or represent compensatory changes to counteract disease progression.

2.1.7.3 DEHYDROASCORBIC ACID TRANSPORT IN THE MITOCHONDRIA

Despite evidence to suggest decreased intracellular glucose levels, circulating glucose and insulin levels have historically been normal in affected individuals.[5] Therefore, the sub-tracellular location of GLUT10 has been closely examined to determine if there may be an alternate pathogenic mechanism of ATS. GLUT10 was found to localize to the mitochondria, specifically in the smooth muscle cells of the aorta as well as insulin-stimulated adipocytes. When stimulated by insulin, GLUT10 localizes to the Golgi apparatus and then to the mitochondria. As glycolysis is localized to the cytoplasm, rather than the mitochondria, and glucose is not used for any known biochemical reactions in the mitochondria, it is likely that GLUT10 does not transport glucose into the mitochondria. Instead, researchers found that GLUT10 transported dehydroascorbate, the oxidized form of vitamin C (ascorbate) to mitochondria, increasing intracellular ascorbate levels.[32] Dehydroascorbic acid is thought to be reduced to ascorbate in the mitochondria and released to the cytoplasm through an as yet unidentified transporter. This putative ascorbate recycling pathway is hypothesized to be especially important in species that lack gulonolactone oxidase, an enzyme responsible for the synthesis of ascorbate. These species, including humans, guinea pigs, and fish, are unable to synthesize their own vitamin C and rely on diet for this essential antioxidant and

enzyme cofactor. This difference in ascorbate metabolism explains the severe vascular manifestations of GLUT10 deficiency in humans[2] and fish[16] but not in mice.[15]

Ascorbate protects cells from reactive oxygen species due to its strong reducing capacity.[41] This hypothesis was supported by reduced mitochondrial dehydroascorbate in higher oxidative stress conditions along with increased reactive oxygen species levels, mitochondrial fragmentation and dysfunction, and increased cell proliferation and migration in aortic smooth muscle cells and in arteries from mice homozygous for a G128E missense mutation in GLUT10,[32,42] which impairs mitochondrial targeting. The G128E mutant mice were consistent with mild arterial disease, only developing increased blood pressure with age as opposed to severe arterial tortuosity.[42]

2.1.7.4 DEHYDROASCORIBIC ACID TRANSPORT IN THE ENDOPLASMIC RETICULUM

Based on the previous findings supporting the possibility of GLUT10 as a dehydroascorbic acid transporter, other researchers have further theorized that GLUT10 may also function in the endoplasmic reticulum. Defective dehydroascorbic acid transport was demonstrated in ATS fibroblast endomembranes, causing researchers to postulate a role for ascorbic acid in the production of extracellular matrix proteins in addition to its mitochondrial function. Ascorbic acid is a cofactor for the prolyl hydroxylases, a group of enzymes involved in the post-translational modification of prolines which are abundant in extracellular matrix proteins including collagen and elastin. Therefore, a defect in transport causing a shortage of ascorbic acid may lead to impaired function of extracellular matrix proteins, which has been widely observed in ATS fibroblasts.[43] Proline hydroxylation has been long known to be essential for the stability of collagen.[44] The functional importance of the hydroxylation of prolines in elastin is less well

understood, however, the non-random nature[45] and disease-association of altered proline hydroxylation have been previously characterized.[46]

2.2 BIOMECHANICAL PROPERTIES OF THE SKIN

The skin's unique biomechanical properties are primarily influenced by the extracellular matrix proteins of the dense irregular connective tissue that make up the dermis, the layer of skin between the epidermis and the subcutaneous tissues. Specifically, this dense irregular connective tissue makes up the reticular region of the dermis, a thick layer that lies below the papillary region, the other layer of the dermis that is comprised of loose areolar connective tissue. Collagen is the main structural component of the reticular dermis comprising around 77% of the weight of the skin, as it is rigid and possesses high tensile strength to resist tearing.[47] Conversely, elastin provides elasticity to connective tissue due to its flexible, resilient properties. During movement, the ground substance, comprised of proteoglycans, is the lubricant between collagen and elastin, but it does not heavily contribute to either the tensile strength or elasticity of the skin.[47] A basement membrane and type VII collagen fibrils anchor the epidermis to the dermis. The primary source of these extracellular matrix proteins are fibroblasts, large, spindle or star-shaped cells that are the main cell type in connective tissue. While skin fibroblasts from individuals affected with ATS have shown significantly reduced and fragmented elastin fibers,[35] any potential effects of this on the biomechanical properties of the skin have yet to be characterized *in vivo*.

2.2.1 VISCOELASTICITY

The elastic property of materials resists deformation in response to external forces and returns the material to its original shape upon removal of the force. Solids generally behave live elastic materials and the stiffness of such materials is quantified by the elastic modulus, also known as Young's modulus. Viscoelasticity also resists against deformation by forces, but the deformation is not reversed in viscous material upon removal of the force. Viscosity is generally the property of liquids. Many natural materials, including the skin, possess both elastic and viscous properties and are hence considered to be viscoelastic. The collagen and elastin fibers which allow for this viscoelasticity have been extensively studied *in vitro* to determine their mechanics, including elastic limit.[48] The time it takes the skin to return to its baseline after being stressed or stretched is referred to as retraction time. The longer the retraction time, the more viscous and less elastic the material is. Retraction time has historically been used as a rapid assessment of acute illnesses, like dehydration, as well as chronic issues with viscoelasticity.[49]

2.2.2 ADDITIONAL QUANTIFIABLE SKIN PROPERTIES

2.2.2.1 HYDRATION

Skin moisture is an important component in many of the skin's primary functions, largely as a protective barrier from exogenous environmental harms as well as its role in thermoregulation. Epidermal hydration is maintained as the stratum corneum, the outermost layer of the epidermis, retains water. The stratum corneum typically consists of ten to thirty percent water, with ten percent being the minimum amount required to maintain its supple and flexible nature.[50] Keratin within the stratum corneum prevents water evaporation. Epidermal hydration is also dependent

upon the maintenance of evaporative loss through water supplied by the bottom layers of the epidermis, the dermis, and the sebaceous glands.[51] Several external factors can greatly influence skin hydration including an individual's water intake and use of skin moisturizers and some intrinsic factors including age and sex have also been implicated.[52] Maintaining skin hydration is essential for cell proliferation and differentiation, and is further increased by pathological processes such as inflammation.[53]

2.2.2.2 DERMAL THICKNESS

Overall skin thickness is influenced by several factors including sex and age and also varies widely between different areas of the body. This is particularly true of epidermal thickness, while dermal thickness remains more consistent throughout different parts of the body, around 1.1 millimeters. Males typically have greater skin thickness than females due to increased collagen density[54] and skin thickness declines with age due to decreased collagen and ground substance synthesis and maintenance.

2.2.3 PREVIOUS CHARACTERIZATIONS

Cutaneous findings, including inelastic skin, have been noted in several connective tissue disorders. *In vivo* studies have demonstrated altered biomechanical properties of the skin specifically in Ehlers-Danlos syndrome, Williams syndrome, scleroderma, and cutis laxa as well as skeletal dysplasias including osteogenesis imperfecta.[48] In individuals affected with Williams syndrome, which presents with stenosis of the great arteries, but no widespread arterial tortuosity, had significantly reduced viscoelastic and elastic moduli compared to healthy controls.[8] In individuals with cutis laxa, a disease characterized by visually lax and loose skin,, he elastic and

viscoelastic moduli are even more markedly reduced than in Williams syndrome. In addition, the retraction time is greatly increased.[9] To date, similar *in vivo* cutaneous studies have not been performed to determine differences between individuals affected with ATS and healthy controls. This study aims to determine what, if any, differences in the quantifiable cutaneous properties of elasticity, retraction time, hydration, and thickness exist among individuals affected with ATS and healthy controls.

3.0 MANUSCRIPT

3.1 BACKGROUND

Arterial tortuosity syndrome (ATS) is a rare connective tissue disorder caused by biallelic mutations in the *SLC2A10* gene, which encodes for a facilitative glucose transporter GLUT10. The characteristic feature of ATS is systemic arterial tortuosity of the medium and large vessels, conferring a high lifetime risk of ischemic and hemorrhagic events, mainly caused by the rupture of aneuryms.[5] There is variable expression of a number of other features, including other cardiovascular anomalies, craniofacial features, and generalized findings characteristic of connective tissue disorders. For example, most individuals affected with ATS exhibit cutaneous findings consistent with other connective tissue disorders.[1] Cutaneous features similarly exhibit variable expression, ranging in severity from velvety, hyperextensible skin to significantly loose or lax skin. In additional to external cutaneous features, histological studies of affected vascular tissue have demonstrated reduced external elastic lamina and fragmented internal lamina.[21] No study has attempted to quantify the effect of these external and internal cutaneous findings on the biomechanical properties of the skin in individuals affected with ATS.

In this study, we sought to determine the functional consequences of systemic connective tissue disease in individuals affected with arterial tortuosity syndrome by measuring physical properties of the skin, including elasticity, viscoelasticity, epidermal hydration, and dermal thickness. We hypothesized that individuals with ATS would have reduced skin elasticity, viscoelasticity, and dermal thickness as well as increased skin retraction time, epidermal hydration, and collagen intensity compared to healthy controls.

3.2 METHODS

To investigate potential differences in biomechanical and quantifiable properties of the skin, we performed several noninvasive *in vivo* skin measurements with a verified instrument, the Coretex DermaLab® Combo, in individuals with ATS and their unaffected relatives. This instrument is equipped with a variety of probes. We utilized the elasticity probe, which measured the skin's elasticity, viscoelasticity, and retraction time, the hydration probe, which measured epidermal hydration, and the ultrasound probe, which measured dermal thickness and collagen intensity. We adjusted these measurements to account for age-related differences and compared our findings among our cases and controls.

3.2.1 HUMAN SUBJECTS

All the participants in this study gave their informed consent for participation in a study examining the etiology and natural history of connective tissue disorders, specifically cutis laxa and related disorders including ATS. All procedures were approved by the Institutional Review Board of the University of Pittsburgh's Office for the Protection of Human Subjects in Research (Appendix A).

Individuals were eligible to participate in this study if possessed a clinical diagnosis of arterial tortuosity or had a first-degree relative who had arterial tortuosity (Appendix B). Participants in this study were recruited and consented in person at the 2017 Arterial Tortuosity Syndrome Annual Conference in Little Rock, Arkansas held by A Twist of Fate-ATS (ATOF). We obtained self-reported ages, genetic information, and family history of participants or from the adult parents of minor participants through. We performed a variety of skin measurements using the DermaLab® Combo SkinLab on each participant as described below.

3.2.2 BIOMECHANICAL SKIN MEASUREMENTS - DERMALAB®

The DermaLab® Combo is an instrument devised to perform rapid, non-invasive, *in vivo* measurements of skin properties. DermaLab® modules have been validated by multiple studies since its introduction by Cortex Technology thirty years ago.[48] All described measurements of the skin were evaluated on four areas of interest - the right dorsal forearm, the right ventral forearm, the left ventral forearm, and the left dorsal forearm - approximately halfway between the wrist and the elbow on each participant, as shown in Figure 2. Combined, measurements take approximately fifteen minutes to complete.

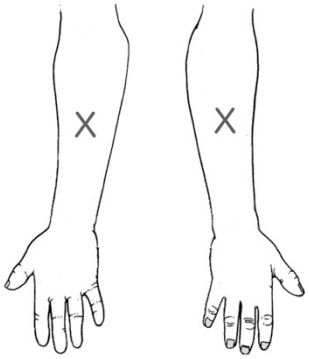

Figure 2. Right arm areas of interest for measurement

3.2.2.1 ELASTICITY

The DermaLab® elasticity module uses a suction chamber applied to the skin using a two-sided adhesive tape ring. It measures the viscoelastic properties of the skin by measuring the stress/strain required to elevate the skin a standardized height of 1.5 mm, where stress/strain is the force per unit area within the skin due to external influence. Elastic modulus (E) is then used to reflect the relationship between the distensibility and elasticity of the skin, specifically stress over strain and is calculated by dividing the change in pressure applied by the change in elevation of the skin,

multiplied by the assumed constant skin thickness. Thus, the provided E in megapascals (MPa) is an indication of the elasticity of the measured skin, with a high E being indicative of stiffer, more sclerotic, or overall less extensible skin while a low modulus indicates stretchy, pliable skin.[48] When measuring elasticity, the DermaLab® also measures retraction time, the amount of time required for the elevated skin to return to its baseline when applied pressure is removed. This value, measured in seconds, is another indicator for the elastic properties of the skin.[55] A longer retraction time is indicative of inelastic, more viscous skin. A viscoelastic modulus (VE) is also calculated using these elasticity and retraction time measurements.[9] In this study, five consecutive rounds of applied pressure/retraction were performed on each area of interest: right forearm, right outer arm, left forearm, and left outer arm.

Figure 3. Elasticity probe

3.2.2.2 HYDRATION

The DermaLab® hydration module utilizes a conductance measurement to determine the water content of the stratum corneum via eight pin electrodes reported in the units of micro Siemens (uS), a measure of electrical conductance. The higher the conductance, the greater the epidermal hydration.[53] In this study, two readings were obtained for each area of interest by placing the probe firmly against the skin.

Figure 4. Hydration pin probe

3.2.2.3 ULTRASOUND

The DermaLab® ultrasound module contains a rotating ultrasound sensor probe with a center frequency of 20 mega Hertz to instantly measure collagen intensity and assess the thickness of the dermis by delineating the border between the epidermis, the dermis, and the subcutaneous fat tissue.[56] Outputs include collagen density measure in megapascals (MPa) and skin thickness measured in millimeters (mm). The image output is also available for analysis. The module is prepared by filling the ultrasound chamber with water, which serves as the conductor, and then covering the water with film, which is placed against the skin covered with a small amount of ultrasound gel. In this study, ultrasound imaging was performed on each area of interest.

Figure 5. Ultrasound probe

3.2.3 STATISTICAL ANALYSIS

The collected measurements were combined for analysis. The multiple readings of each parameter were averaged and the left and right arm readings for each arm were also averaged to provide a single ventral and dorsal measurement for each parameter. Thus, data was analyzed to look for statistically significant differences between cases and controls, separately for inner and outer arm. The rationale for separate analysis of the ventral and dorsal measurements is that the dorsal arm is generally more exposed to the sun. Sun damage is a major factor in the age-related deterioration of the skin mechanics.[57] All statistical analysis was performed using R software.

The Shapiro-Wilkes test for normality was performed on each parameter to ensure a normal distribution for further analysis. All properties were normally distributed, except for retraction time which was skewed right on both dorsal and ventral measurements. Chi-squared tests were performed on the categorical age and sex demographic date and t-tests were used for the continuous biomechanical property data. Pearson's correlations were calculated between age and

each biomechanical property, excluding retraction time, for which Spearman's test was used. Linear regression was utilized to correct each parameter for the confounding factor of age.

3.3 RESULTS

The participants in this study included eight individuals affected with ATS and utilized twenty-one of their unaffected relatives as controls. While sex distribution was not statistically significant between cases and controls, the population affected with ATS was significantly younger than controls (Table 1). The ages of the cases ranged from 6 years of age to 56 years of age while the ages of the controls ranged from 9 years old to 69 years old. All of our participants were Caucasian.

Table 1. Participant demographics

	Affected (n=8)	**Unaffected (n=21)**	**P-value**
Age	16.1 ± 6.6 years	36.2 ± 4.2 years	0.009 (t-test)
Sex (% male)	63.5%	42.9%	0.793 (Chi-squared)

Four of our eight affected individuals reported mutation status by providing copies of previous genetic testing reports. All of these participants were compound heterozygotes for *SLC2A10* pathogenic variants, with the known recurrent c.394C>T or p.Arg132Trp mutation being the most frequently observed among participants occurring in two of the cases (Table 2). We also included four individuals of unknown *SLC2A10* genotype by self-report, although three of them report undergoing genetic testing and having a molecular diagnosis. One affected participant had been clinically but not molecularly diagnosed.

Table 2. Known *SLC2A10* genotypes of cases

	Mutation One		Mutation Two	
Reference Sequence	**Nucleotide**	**Amino Acid**	**Nucleotide**	**Amino Acid**
NM_030777.3	c.394C>T	p.R132W	c.4+5G>A	NA
NM_030777.3	c.314C>T	p.R105H	c.727C>A	p.Q234K
NM_030777.3	c.394C>T	p.R132W	c.800delC	NA
NM_030777.3	c.848C>A	p.A283D	c.2381G>A	p.R794H

Pearson and Spearman tests were performed to determine the correlation of each cutaneous measure with age (Table 3). Dermal hydration, collagen intensity, and VE were negatively correlated with increasing age while dermal thickness and retraction time were positively correlated with age. The only statistically significant correlations were between age and the collagen intensity measured on the dorsal forearm and between age and the retraction time measured on the dorsal forearm. This suggests that age truly has an effect on collagen density, more so on the dorsal forearm than the ventral forearm, as well as on retraction time, again more so on the dorsal forearm than the ventral forearm.

Table 3. Correlation of age and parameters

	Ventral Forearm		Dorsal Forearm	
	Correlation	**P-value**	**Correlation**	**P-value**
Elastic modulus (MPa)	0.157	0.415	0.143	0.461
Viscoelastic modulus (MPa)	-0.320	0.0903	-0.432	0.0193
Retraction Time (s) *	0.380	0.0421	0.575	0.00111
Hydration (μS)	-0.0622	0.749	-0.252	0.188
Skin Thickness (mm)	0.303	0.124	0.253	0.212
Collagen Intensity (MPa)	-0.408	0.0348	-0.608	0.000979

*Spearman's method

Before performing regression analysis to correct for the age differences in the data set, t-tests were performed on the means of each parameter to identify differences between cases and controls. Cases exhibited significantly lower E (ventral p-value = 0.0005513, dorsal p-value = 0.00582) than controls. Cases also exhibited significantly increased dorsal forearm collagen intensity (p-value 0.04646), although the same statistically significant difference was not noted on the ventral forearm. No other parameter was significantly different between cases and controls, although there were trends among the dataset. Cases exhibited reduced viscoelastic modulus (VE), increased hydration, reduced dermal thickness, and increased collagen intensity when compared to controls (Table 4, Table 5, Figure 6, Figure 7).

Table 4. Biomechanical properties of the ventral forearm

	Ventral Forearm		
	Affected	**Unaffected**	**P-vale**
Elastic modulus (MPa)	3.777 ± 0.2	4.342 ± 0.2	0.000551
Viscoelastic modulus (MPa)	7.533 ± 0.8	8.4747 ± 1.1	0.324
Retraction Time (s)	132.8 ± 9.7	132.6 ± 9.7	0.984
Hydration (μS)	119.6 ± 22.3	112.803 ± 13.9	0.619
Skin Thickness (mm)	979.3 ± 180.0	1024.2 ± 145.1	0.574
Collagen Intensity (MPa)	58.50 ± 12.0	53.19 ± 8.6	0.385

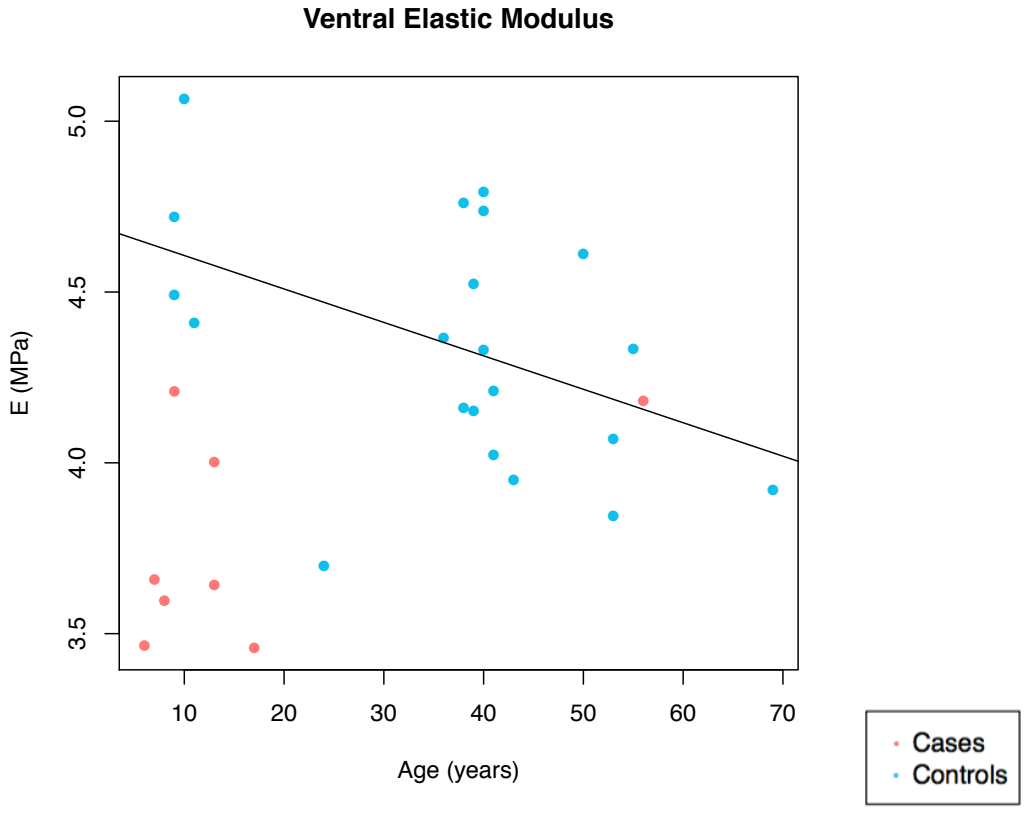

Figure 6. Scatterplot of the ventral elastic modulus data

Table 5. Biomechanical properties of the dorsal forearm

	Dorsal Forearm		
	Affected	**Unaffected**	**P-value**
Elastic modulus (MPa)	3.959 ± 0.3	4.411 ± 0.1	0.00582
Viscoelastic modulus (MPa)	7.371 ± 0.9	8.236 ± 0.9	0.304
Retraction Time (s)	139.0 ± 16.5	152.8 ± 24.7	0.523
Hydration (uS)	92.78 ± 17.7	89.32 ± 11.7	0.760
Skin Thickness (mm)	1009.8 ± 311.2	1262.1 ± 219.5	0.534
Collagen Intensity (MPa)	37.04 ± 6.8	34.12 ± 5.6	0.0464

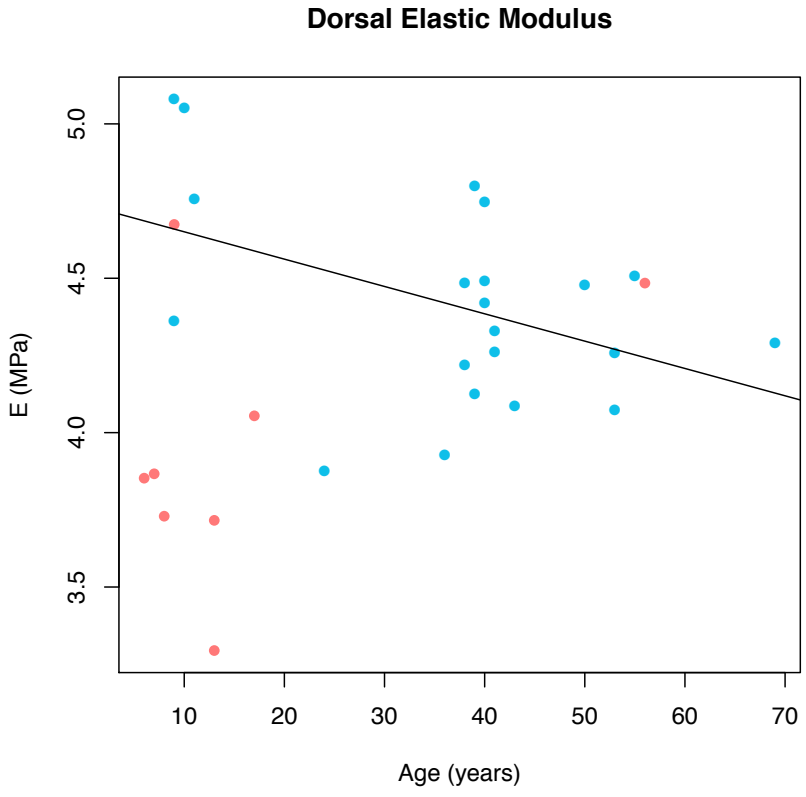

Figure 7. Scatterplot of the dorsal elastic modulus data

Linear regression analysis was then performed on each parameter to adjust for the known confounding factor of age, as age was shown to be statistically significant between cases and controls. Cases exhibited significantly lower E (ventral p-value = 0.0005513, dorsal p-value = 0.00582) and VE (ventral p-vale = 0.02107, dorsal p-value = 0.003055) than controls. No other parameter was significantly different between cases and controls. The trends previously noted among the other parameters were not static. Cases exhibited reduced dorsal dermal thickness, but ventral dermal thickness was increased over controls. Also, cases demonstrated increased dorsal collagen intensity when compared to controls but decreased ventral collagen intensity (Table 6 and Table 7). Cases also exhibited increased skin retraction time, which had not been previously apparent prior to linear regression (Table 6 and Table 7). When adjusted for age, the epidermal hydration remained increased in the affected population on the ventral forearm, but was lower in the affected population compared to the unaffected population on the dorsal forearm (Table 6 and Table 7).

Table 6. Age-adjusted biomechanical properties of the ventral forearm

	Ventral Forearm		
	Affected	**Unaffected**	**P-value**
Elastic modulus (MPa)	3.852 ± 0.2	4.515 ± 0.2	0.0005
Viscoelastic modulus (MPa)	8.635 ± 1.0	11.005 ± 1.0	0.021
Retraction Time (ms)	120.9 ± 8.4	105.2 ± 8.5	0.073
Hydration (μS)	120.1 ± 15.9	113.9 ± 16.1	0.703
Skin Thickness (mm)	925.9 ± 88.5	907.6 ± 90.2	0.838
Collagen Intensity (MPa)	63.83 ± 6.5	64.84 ± 6.6	0.878

Table 7. Age-adjusted biomechanical properties of the dorsal forearm

	Dorsal Forearm		
	Affected	**Unaffected**	**P-value**
Elastic modulus (MPa)	4.013 ± 0.2	4.535 ± 0.2	0.00659
Viscoelastic modulus (MPa)	8.613 ± 0.8	11.09 ± 0.8	0.00306
Retraction Time (ms)	106.6 ± 19.3	78.25 ± 19.5	0.154
Hydration (μS)	99.65 ± 12.8	105.1 ± 13.0	0.674
Skin Thickness (mm)	1071.3 ± 198.6	1079.7 ± 189.8	0.966
Collagen Intensity (MPa)	46.79 ± 3.9	43.95 ± 3.8	0.471

While our cases ranged in age from 6 years to 56 years, the average age of the cases was 16.1 years and the median age of the cases was 11 years. The 56-year-old case was the only participant affected with ATS over the age of 20. This individual was also the only case that did not possess a molecular diagnosis in addition to a clinical diagnosis. We performed a sensitivity analysis removing this case from the data set and repeating analysis to account for the possibility that this individual's findings may have had a disproportionate effect on our findings. The differences in retraction time between cases and controls was statistically significantly (ventral p-value 0.0482, dorsal p-value 0.00449) after this participant was removed from analysis (Table 8 and Table 9). The contradictory trends seen in epidermal hydration and collagen intensity resolved, with cases now demonstrating both slightly reduced hydration and slightly reduced collagen intensity when compared to controls, although this difference continued to not be significant (Table 8 and Table 9). The contradictory trends between dorsal and ventral dermal thickness persisted (Table 8 and Table 9).

Table 8. Age-adjusted biomechanical properties of the ventral forearm with outlier removed

	Ventral Forearm		
	Affected	**Unaffected**	**P-value**
Elastic modulus (MPa)	4.23 ± 0.2	4.70 ± 0.2	0.000057
Viscoelastic modulus (MPa)	8.50 ± 1.1	11.80 ± 1.1	0.00611
Retraction Time (ms)	121.58 ± 9.9	101.11 ± 9.6	0.0482
Hydration (μS)	118.50 ± 18.7	122.90 ± 18.1	0.815
Skin Thickness (mm)	930.35 ± 105.64	872.32 ± 103.92	0.588
Collagen Intensity (MPa)	63.08 ± 7.3	71.32 ± 7.2	0.268

Table 9. Age-adjusted biomechanical properties of the dorsal forearm with outlier removed

	Dorsal Forearm		
	Affected	**Unaffected**	**P-value**
Elastic modulus (MPa)	3.98 ± 0.2	4.74 ± 0.2	0.000476
Viscoelastic modulus (MPa)	8.49 ± 0.8	11.82 ± 0.8	0.000596
Retraction Time (ms)	108.20 ± 22.8	68.9651 ± 22.1	0.00449
Hydration (μS)	99.01 ± 15.3	108.1 ± 14.9	0.674
Skin Thickness (mm)	1059.5 ± 327.2	1075.6 ± 222.1	0.947
Collagen Intensity (MPa)	46.21 ± 4.6	46.67 ± 4.3	0.923

3.4 DISCUSSION

In this study, we evaluated elastic modulus (E), viscoelastic modulus (VE), and retraction time as well as epidermal hydration, dermal thickness, and collagen intensity among individuals affected with ATS. We hypothesized that individuals with ATS would exhibit differences in these properties when compared with their unaffected relatives, namely reduced E, VE, and dermal thickness with increased retraction time, epidermal hydration, and collagen intensity. Our sample consisted of eight individuals clinically diagnosed with ATS ranging in age from 6 years old to 56 years old. Of these eight individuals, seven have been molecularly diagnosed with biallelic *SLC2A10* pathogenic variants. Of note, one affected individual was significantly older than the other affected individuals at 56 years of age and this participant was the one case that did not have a molecular diagnosis confirming biallelic *SLC2A10* pathogenic variants. Twenty-one unaffected relatives of our affected participants ranging in age from 9 years old to 69 years old were used as our healthy control group.

Our affected population exhibited significantly lowered elastic modulus (E) compared to the unaffected population. Age is not expected to significantly influence skin elasticity until the age of 70,[58,59] which was reflected in the lack of correlation of E and participant age in our data set. This finding was particularly robust, as the difference in E was maintained when adjusted for age as well as when the outlier was removed from analysis. Cases also exhibited reduced viscoelastic modulus (VE) when compared to healthy controls. Correlation between age and VE was statistically significant on the dorsal forearm, which was reflected in the difference in VE becoming statistically significant after age-adjustment was performed. This difference was maintained when the outlier was removed from analysis. The correlation between age and

retraction time was also statistically significant, both dorsally and ventrally. Cases exhibited increased retraction time compared to controls when the outlier was removed from analysis.

The skin of individuals affected with ATS was described as hyperextensible,[11] consistent with our results in reduced E and VE. Qualitative observations also noted that ATS skin returned to baseline without issue.[11] Our quantitative results, however, do show that the amount of time required for the skin to retur to baseline is increased over individuals without ATS, indicating that the sensitivity to detect alterations in skin mechanics in quantitative measurements is higher than the sensitivity of qualitative observations. Taken together, reduced E and VE with increased retraction time are all consistent findings with dysregulation of the extracellular matrix affecting typical biomechanical functioning of the skin.

Previous studies investigated the biomechanics of two elastic fiber disorders, cutis laxa[9] and Williams syndrome[8,46] with similar findings to our results in ATS. In cutis laxa (CL), affected individuals also demonstrate reduced E and VE and increased retraction time.[9] However, the increased retraction time demonstrated in our cases was not as robust as in CL, and the magnitude of increase in mean retraction time relative to controls was uch less in ATS (20 ms, 20%) than in CL (530 ms, 85%), consistent with more severely inelastic skin in CL than in ATS. In studies examining biomechanical skin properties in Williams syndrome, affected individuals exhibited reduced E and VE with about the same effect size as our observations in ATS, but no significant difference was found in the retraction time of individuals with Williams syndrome.[8] Therefore, our findings suggest a skin phenotype in ATS that is less severe than CL, and the same or slightly more severe than Williams syndrome. Given the prior evidence of altered alstic fiber structure and function in all three diseases, we can conclude that the reduction of E and VE with a possible

additional increase RT are shared characteristics of a skin phenotype caused by genetic disorders of elastic fibers.

Our affected participants did not demonstrate a significant difference in epidermal hydration compared to the unaffected group. Currently there are no reports in the literature that measured hydration in related connective tissue disorders. However, a mouse model of autosomal recessive CL 1C (ARCL1C, also known as LTBP4-related CL) did show increased epidermal hydration, which was interpreted as a signed of impaired skin barrier function.[60] The lack of significant and consistent change in epidermal hydration in our affected participants supports the conclusion that the epidermal barrier function is preserved in ATS.

We predicted that individuals with ATS would have reduced thickness based on findings of other connective tissue disorders, namely Ehlers-Danlos syndrome, in which affected individuals are often reported to have thin, fragile, and translucent skin.[61] However, we did not observe a significant difference in this parameter. These findings correlate with the phenotype of ATS, which does not suggest markedly different skin thickness in individuals affected with ATS. There have not been reports of thin skin with visible vessels or easy bruising.

Finally, we predicted that individuals with ATS may exhibit increased collagen intensity due to a recent study reporting that individuals with ATS experience increased collagen deposition in their arteries.[1] While the mechanism of increased collagen deposition is not well understood, general dysregulation of the extracellular matrix has already been implicated in ATS. Moreover, increased collagen deposition has been implicated in thoracic aortic dissection due to collagen's rigidity lending to reduced aortic distensibility.[62] In this study, the affected population did not exhibit significantly different collagen intensity compared to the unaffected population, which may reflect tissue-specific differences in collagen deposition.

This study has several limitations. First, there are limitations based upon our chosen instrument, the DermaLab® Combo SkinLab. This instrument assumes a uniform skin thickness of 1 mm when calculating the elasticity modulus and viscoelastic modulus. This research did not identify a significant difference in skin thickness, which suggests that E and VE would not have been significantly impacted by this issue.

Additionally, due to the small number of affected individuals in our sample, our results must be confirmed in a larger control sample size. Due to restraints from our study protocols, we were unable to collect measurements on individuals who are not related to an affected proband. However, at this time, unaffected carriers of ATS are not known to exhibit clinical findings of ATS and are considered asymptomatic.[11] The relatedness of the cases and controls may have influenced the results of this study due to yet unknown heritable contribution to the parameters tested. In future studies, genotyping unaffected individuals will be important. Finally, due to the nature of our study procedures as well as the severity of ATS, our cases were significantly younger than our controls. A larger sample, with unrelated cases and controls as well as significantly younger controls to match case age, would also allow for expanded analysis of results, including comparing the different skin properties to identify relationships and determining which feature possesses the strongest indication for the presence of ATS. Other avenues for future research include examining genotype-phenotype relationships between specific mutations and cutaneous properties. Despite the presence of several recurrent mutations within the ATS population, no genotype-phenotype correlations have been elucidated.[11]

We would like to compare the results of this study to other research performed in our laboratory examining biomechanical properties of the skin in the related connective tissue disorder cutis laxa. Our previous research was limited to elasticity, viscoelastic modulus, and retraction

time but has recently expanded to include all the parameters measured in this study.[9] Examining differences between individuals affected with ATS, individuals affected with CL, and healthy controls may further elucidate phenotypic differences not only between individuals with a connective tissue disorder and healthy controls, but also between different connective tissue disorders. This may be expanded to compare to other connective tissue disorders, for example, these skin characteristics have also been studied in Williams syndrome.[8]

3.5 CONCLUSIONS

Among our participants, individuals with ATS exhibited significantly reduced cutaneous elastic modulus and viscoelastic modulus when compared to their unaffected relatives. The skin of affected individuals also demonstrated increased retraction time. The differences in epidermal hydration, dermal thickness, and collagen intensity between populations were not significantly different between affected and unaffected individuals. While this interpretation is limited by both the small sample size as well as the relatedness of our cases and controls, biomechanical and other quantifiable skin properties have never been characterized in this rare connective tissue disorder. These results have the potential to aid in diagnosis and management of the ATS population.

4.0 RESEARCH SIGNIFICANCE TO PUBLIC HEALTH AND GENETIC COUNSELING

Rare diseases are defined in the United States as conditions that affect fewer than 200,000 individuals.[63] These conditions like ATS profoundly impact the lives of affected individuals and their family members. While the number of affected individuals may be small within each rare disease, around ten percent of Americans are affected by one of 7,000 defined conditions classified as a rare disease.[64] That is, individually, rare diseases may not appear to be a large component of public health, but taken together, these diseases affect a significant percent of individuals living in the United States.

The ten essential public health services certainly apply to rare diseases. Both the diagnosis of disease and ensuring that affected individuals are linked with trained specialists are particularly applicable.[65] Studies that learn more about the etiology and natural history of rare diseases improve our overall understanding of these conditions in an effort to better diagnose and enhance our ability to treat them.[66] The results of this study help us identify additional phenotypic features of ATS, particularly in determining phenotypic variation among affected individuals. Increasing our understanding of the cutaneous features of ATS can aid in future diagnosis of affected individuals, especially because ATS is likely both underdiagnosed and misdiagnosed in the general population.[5] The issue of proper diagnosis is especially prevalent within the rare disease community. Often, patients who suffer from rare diseases undergo an extended period of time during which they are experiencing symptoms but have yet to be diagnosed, a journey so characteristic of rare diseases that this period has been named the diagnostic odyssey.[67]

The instrument used in this study, the DermaLab® Combo SkinLab, has been verified by several different projects for several different genetic disorders. By continuing to define its abilities, this instrument may one day move from a purely research-based application to widespread use in clinical practice with the ability to aid in the diagnosis of complex connective tissue disorders. Early, accurate diagnosis aids not only in early implementation of management, but also allows health care practitioners, including genetic counselors, to identify other family members at risk, provides relief to the patient and family, and offers the opportunity for these individuals to join disease support networks.[63]

Implementation of management ensures that affected individuals receive appropriate medical treatment by trained specialists. ATS, like many other genetic disorders, is often treated by a multidisciplinary team to address the significant cardiovascular issues as well as the variable other systemic features, including skeletal, ocular, and craniofacial. This treatment approach has been utilized not only among other connective tissue disorders similar to ATS,[68,69] but widely across genetic disorders due to their complex and systemic nature. This study's contribution to the definition of ATS's phenotype allows for the appropriate specialists, including dermatologists and genetic counselors, to be included on these individuals care team. Genetic counselors are uniquely skilled individuals who can guide individuals through a diagnosis of a genetic disease, including shortening an individual's diagnostic odyssey as well as providing anticipatory guidance once a diagnosis has been provided. Once a rare disease has been better defined, elucidating the etiology of a rare disease can further guide diagnosis and provides an avenue for the development of targeted therapies.

Additionally, while the systemic tortuosity of ATS is rare, isolated arterial kinking is more common in the population and may often be reported as a benign finding upon imaging with

increasing tortuosity increasingly indicative of pathology.[70] Isolated arterial tortuosity has been described in nearly all vessel branches in the body with aging, hypertension, atherosclerosis, and diabetes mellitus.[71] Arterial tortuosity has also been described in patients with sickle cell disease[72] and has been implicated in cases of pediatric arterial ischemic stroke.[73] The cardiovascular outcomes of arterial tortuosity syndrome are also more common in the general population. Due to risk factors like tobacco use and hypertension, aneurysm occurs in around one in fifty individuals in the United States. Gaining a deeper understanding about the etiology of both arterial tortuosity and the cardiovascular outcomes associated with ATS will allow for wider understanding of these phenomena in the general population.

APPENDIX A: IRB APPROVAL LETTER

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Zsolt Urban, PHD
From: Margaret Hsieh, MD, Vice Chair
Date: 1/26/2017
IRB#: REN17010069 / PRO10020125
Subject: Genetics of Extracellular Matrix in Health and Disease

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The Renewal for the above referenced research study was reviewed and approved by the Institutional Review Board, Committee G, which met on 1/18/2017.

Please note the following information:

The risk level designation is Greater Than Minimal.

Approval Date: 1/18/2017
Expiration Date: 1/17/2018

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX B: ELIGIBILITY CRTIERIA

Participant ID _____

Family ID _____

Phone Screening Script for
Genetics of Extracellular Matrix in Health and Disease

Telephone Screen Results:

___ Agreed and Eligible ___ Refused ___ Not eligible

_____
Printed Name of Participant

_____ _____
Printed Name of Person Screening Date and Time of Screen

Notes: _____
_____
_____
_____

Script ----------

"Hello. My name is _____ and I am researcher with the University of Pittsburgh Department of Human Genetics. May I please speak with _____?"

{If the potential participant is not available}
"OK, thank you. I'll call back at another time. Is there a better day or time to reach them?"

{If yes} Document here: _____
"Great. I'll call back then. Thank you. Goodbye."
{If no} *"OK. Thank you. Goodbye."*

{If the potential participant is available on the phone}
"Hello. Again, my name is _____ and I am a researcher at the University of Pittsburgh. I work on studies of cutis laxa. You expressed interest in participating in our study about looking at the genetic causes of cutis laxa.

May I talk to you today about our study that is looking at cutis laxa causing genes?"

{If no}: *"OK. Thank you very much for your time. Have a nice day."*

{If yes}: ***"OK. In this study, we are looking at several genes that have been shown to cause cutis laxa. We are interested in using clinical and laboratory information to find disease-causing changes in known genes and to identify new cutis laxa genes. We also will study the effects of the gene changes and how they cause cutis laxa.***

Are you interested in hearing more about the study?"

{If no}: *"OK. Thank you very much for your time. Have a nice day."*

{If yes}: ***"Great! But before enrolling people in the study, we need to make sure that you are eligible. What I would like to do now is ask you a series of questions about your or your family member's health and medical history, specifically pertaining to the skin. All information that I receive from you today by phone will be strictly confidential and stored under lock and key in our files to be reviewed only by our research staff. The purpose of these questions is to determine if you are eligible for our study, and if you are found not to be eligible the information will be destroyed. Your participation in this interview is voluntary; you do not have to answer these questions, and can choose to stop answering these questions at any time.***

Do I have your permission to ask you these questions now?"

No ___ Yes ___

_____ _____
Printed Name of Person Obtaining Consent Role in Research Study

_____ _____
Signature of Person Obtaining Consent Date and Time of Consent

____ All questions were answered to the participant's satisfaction:

Document here:

_____
_____
_____
_____

____ Participant had no questions.

{If no}: *"OK. Thank you very much for your time. Have a nice day."*

{If yes}: ***"Great! If at any time you have questions, please stop me and ask your question.***

1) *"Have you or a family member ever been diagnosed with cutis laxa, has cutis laxa been suspected in you or a family member, or have you or a family member had genetic testing that identified a change in a cutis laxa related gene?"*

___ (A) Yes, cutis laxa has been diagnosed in participant

*"Who diagnosed you with cutis laxa?"*_____

"How old were you when diagnosed?" _____

___ (B) Yes, cutis laxa has been suspected in participant

"Who suspected that you have cutis laxa?" _____

"How old were you when cutis laxa was suspected?" _____

___ (C) Yes, cutis has been diagnosed in a participant's family member

"How are you related to your family member who has cutis laxa?" _____

___ (D) Yes, cutis laxa has been suspected in family member

"How are you related to your family member who is suspected to have cutis laxa?"
_____

___(E) Yes, genetic testing has identified a variant (change) in a cutis laxa related gene in participant

"What gene contained the change?" _____

"What type of genetic testing found the change?" _____

___(F) Yes, genetic testing has identified a variant (change) in a cutis laxa related gene in participant's family member

"What gene contained the change?" _____

"What type of genetic testing found the change?" _____

"How are you related to your family member with the cutis laxa related genetic change?" _____

___ (G) No. Continue with question #2.

2) *"I am now going to ask you a few questions about [your/your family member's] skin:*

[Do you/ does your family] have:

(A) *Loose, lax skin*	___ Yes	___ No
(B) *Skin in redundant folds*	___ Yes	___ No
(C) *Inelastic (doughy) skin*	___ Yes	___ No

sorry to tell you, but you are not eligible for our study. We do appreciate your time today on the phone. Thank you. Goodbye."

For subjects who have passed the screening questions: *"Based on the questions I have asked, you are eligible for the study.*

"The next step is to provide you with all the relevant information you will need to make an informed decision about participating in the study. Do you have the packet you received [in the mail / from your physician] called 'Consent to Act as a Participant in a Research Study'?"

{If no}: "OK. What we can do is send you another packet, and you can contact us once you have received the packet."

{If yes}: "This will take about 15 minutes to go through, do you have enough time right now?"

{If yes}: Move onto "Informed Consent Script for Genetics of Extracellular Matrix in Health and Disease"

{If no}: "When will be a better time to contact you to go through this packet?"

Contact date and time: _____

"*Before going, I would like to ensure that we have your correct contact information:*

Name: _____

Contact Address: _____

_____

_____

_____

Contact Telephone: _____

Best time to call: _____

Contact Email: _____

Thank you very much for your time. Have a nice day."

APPENDIX C: SUPPLEMENTARY DATA

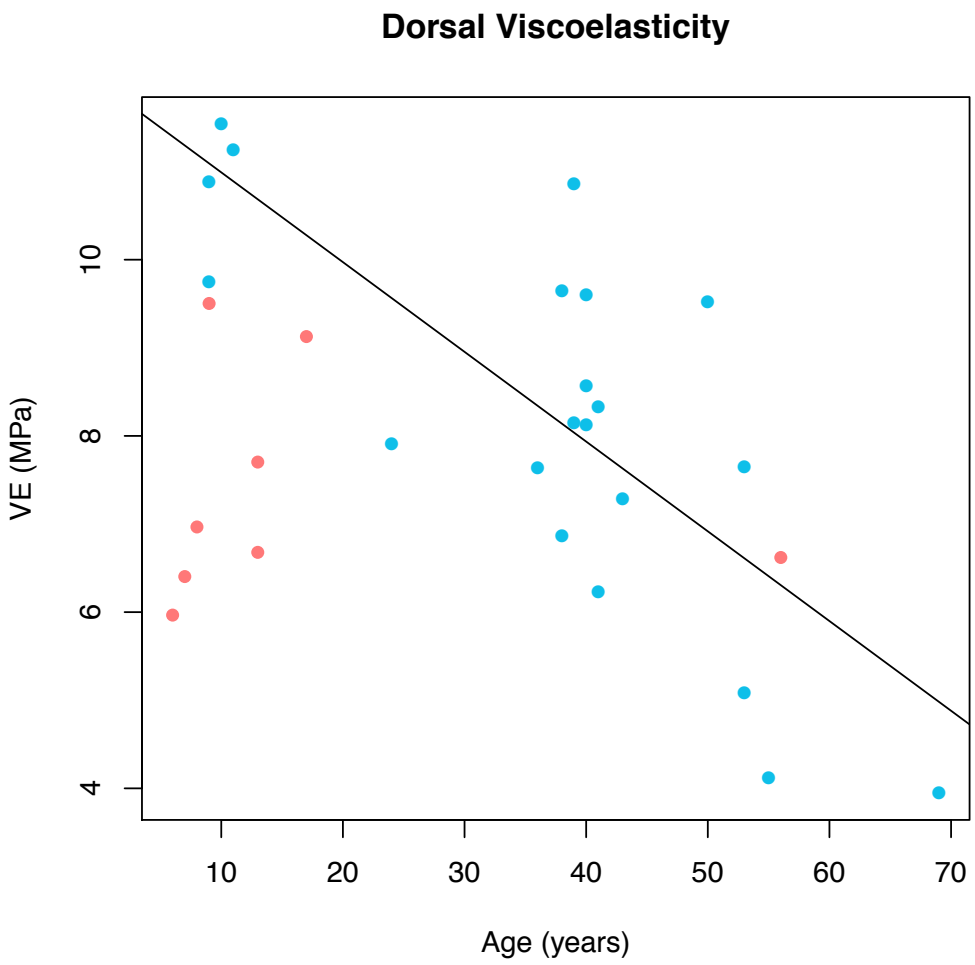

Figure 8. Scatterplot of dorsal viscoelasticity data

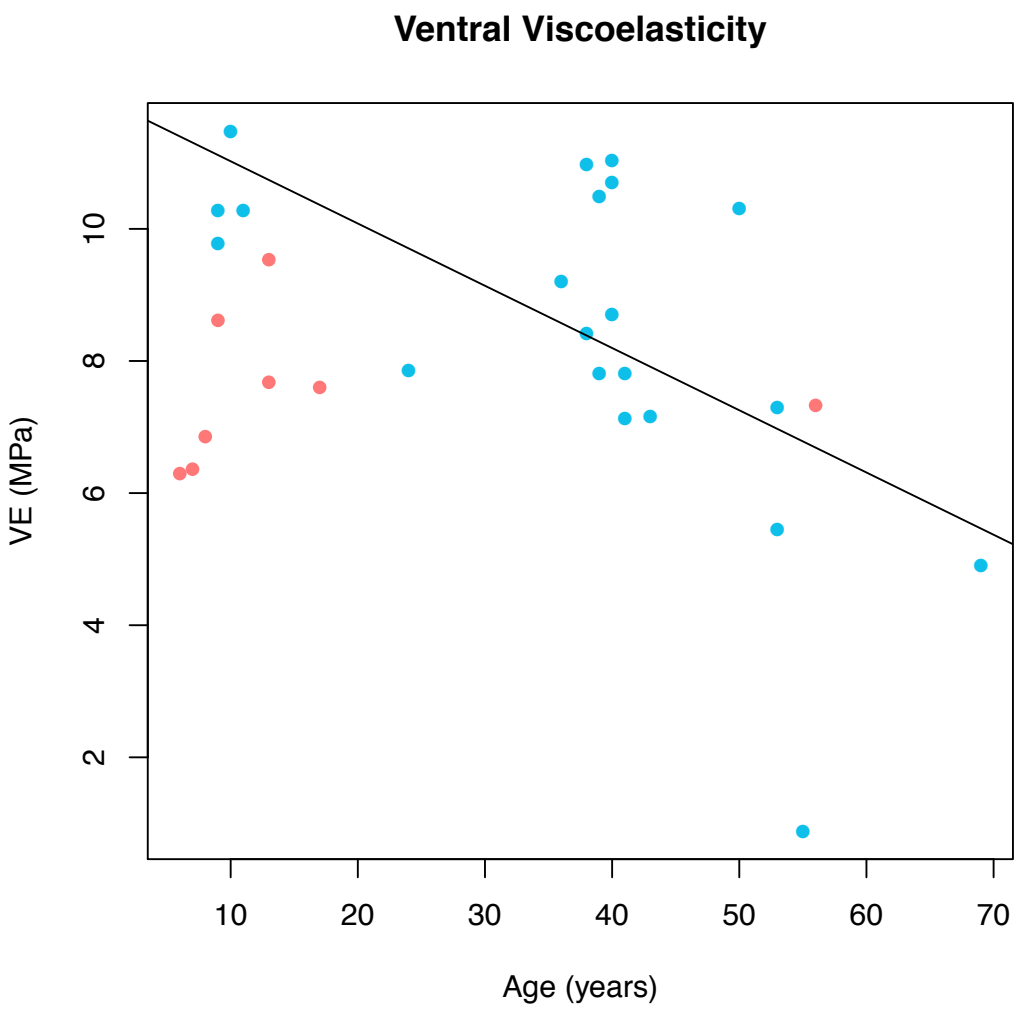

Figure 9. Scatterplot of ventral viscoelasticity data

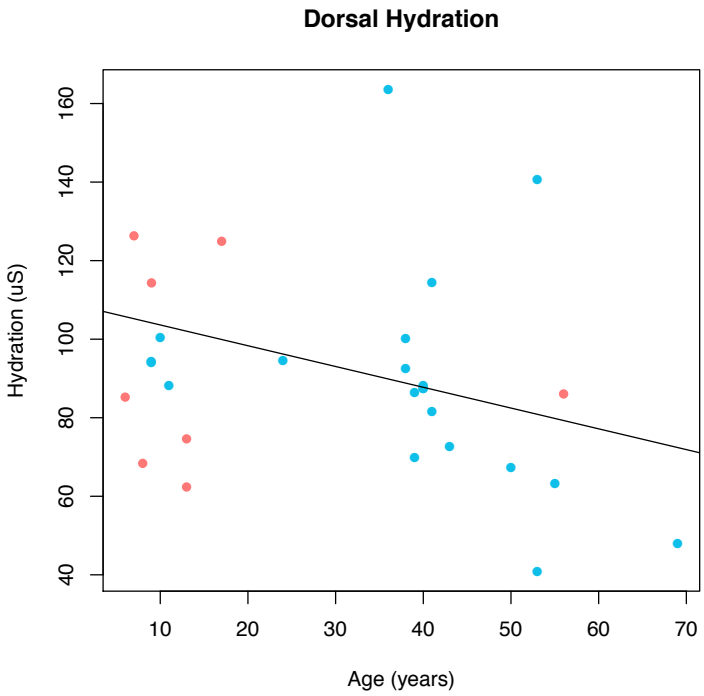

Figure 10. Scatterplot of dorsal epidermal hydration data

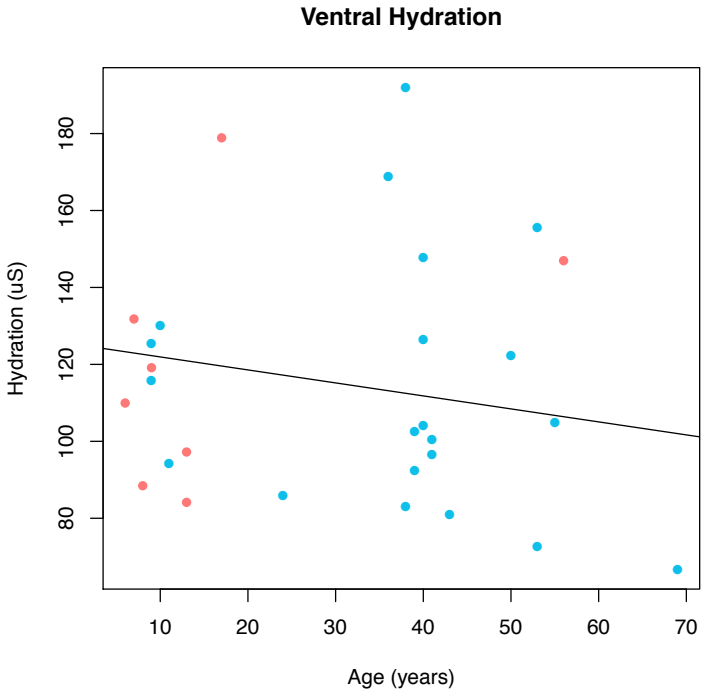

Figure 11. Scatterplot of ventral epidermal hydration data

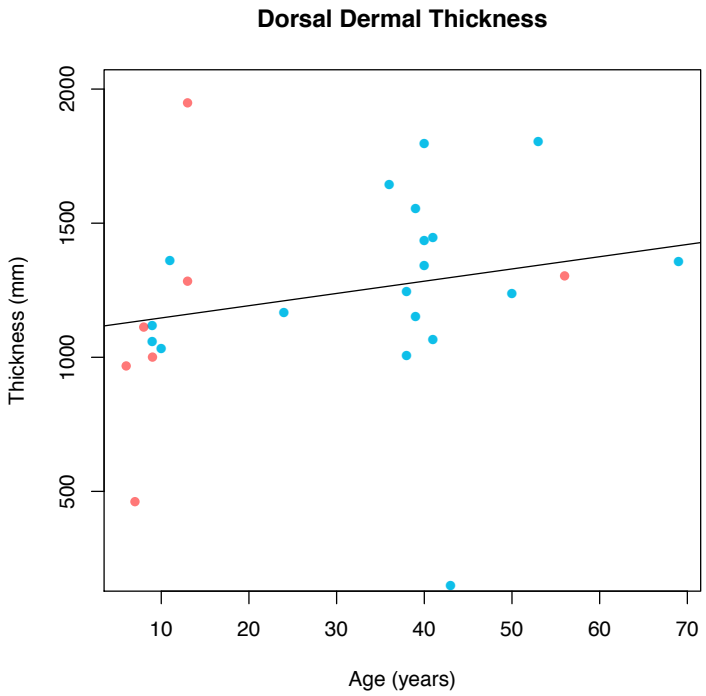

Figure 12. Scatterplot of dorsal dermal thickness data

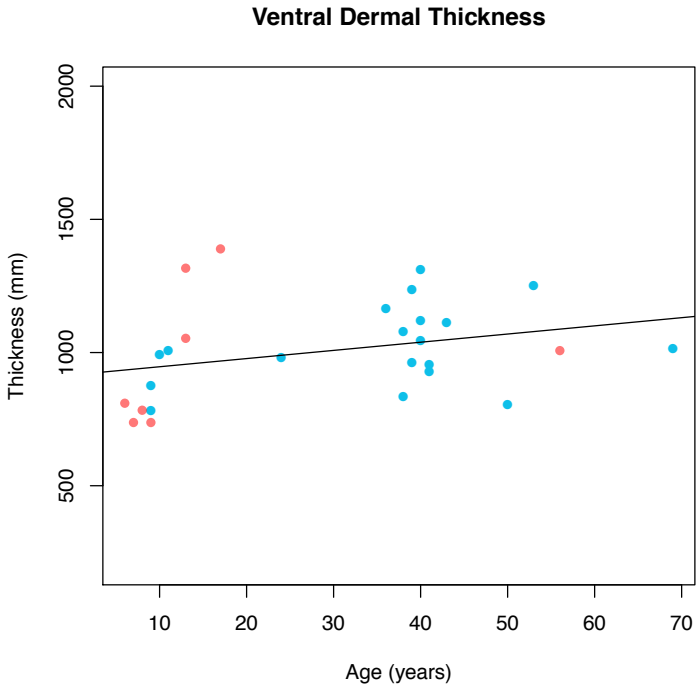

Figure 13. Scatterplot of ventral dermal thickness data

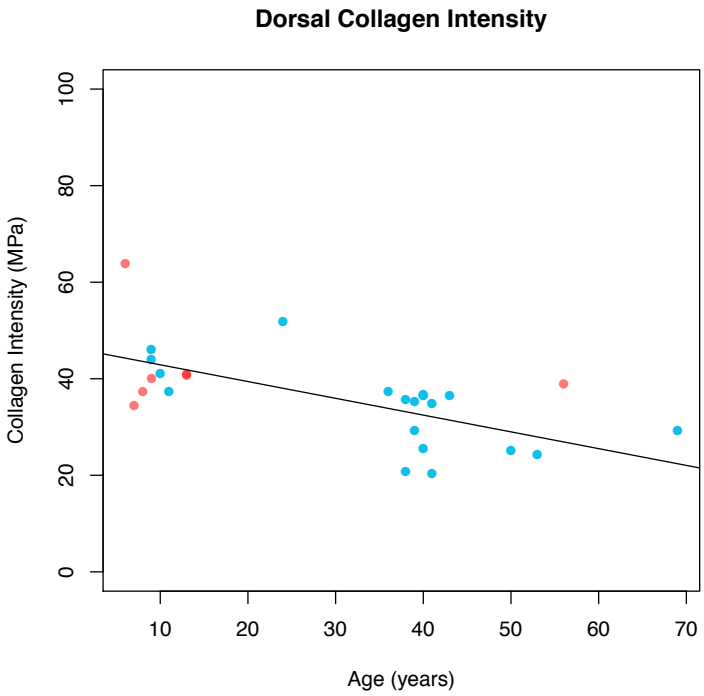

Figure 14. Scatterplot of dorsal collagen intensity data

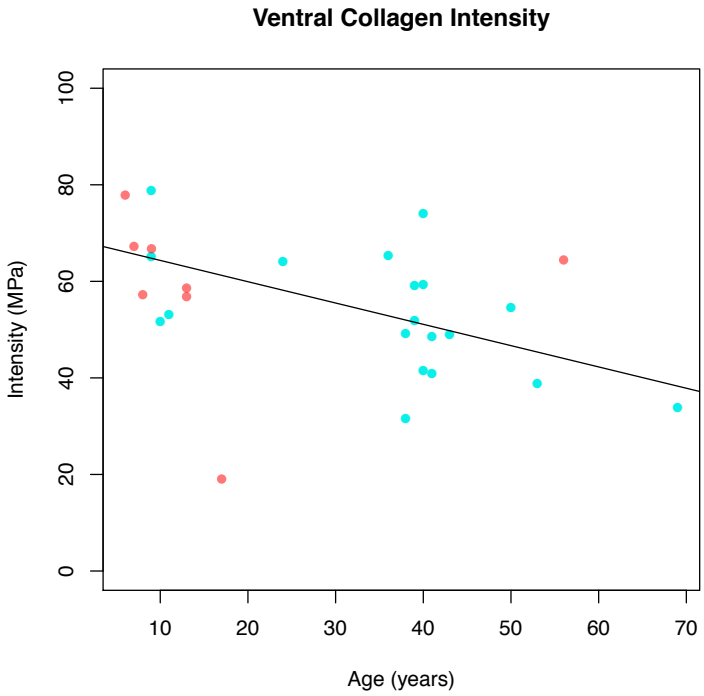

Figure 15. Scatterplot of ventral collagen intensity data

Table 10. Ventral retraction time

	Affected	Unaffected	P-value
RT1	129.6 ± 7.8	128.0 ± 8.2	0.83
RT2	130.6 ± 8.7	130.1 ± 9.3	0.95
RT3	132.5 ± 9.7	133.4 ± 10.6	0.93
RT4	134.6 ± 10.8	136.4 ± 12.3	0.87
RT5	136.7 ± 11.9	135.3 ± 10.0	0.88

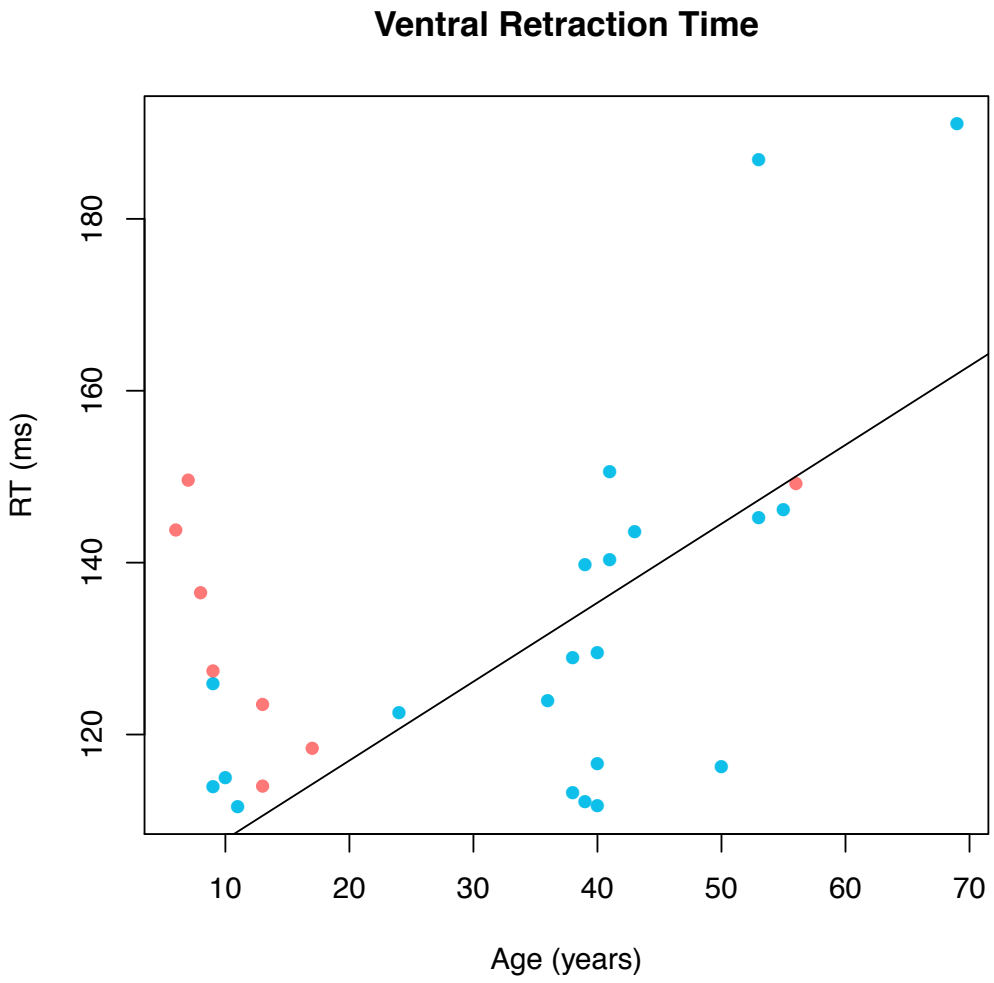

Figure 16. Scatterplot of ventral retraction time data

Table 11. Dorsal retraction time

	Affected	Unaffected	P-value
RT1	131.3 ±8.3	137.0 ± 13.7	0.63
RT2	133.8 ± 11.5	144.0 ± 18.9	0.53
RT3	138.3 ± 16.1	152.4 ± 24.1	0.50
RT4	143.3 ± 21.3	161.1 ± 30.2	0.50
RT5	148.6 ± 27.1	169.4 ± 36.9	0.52

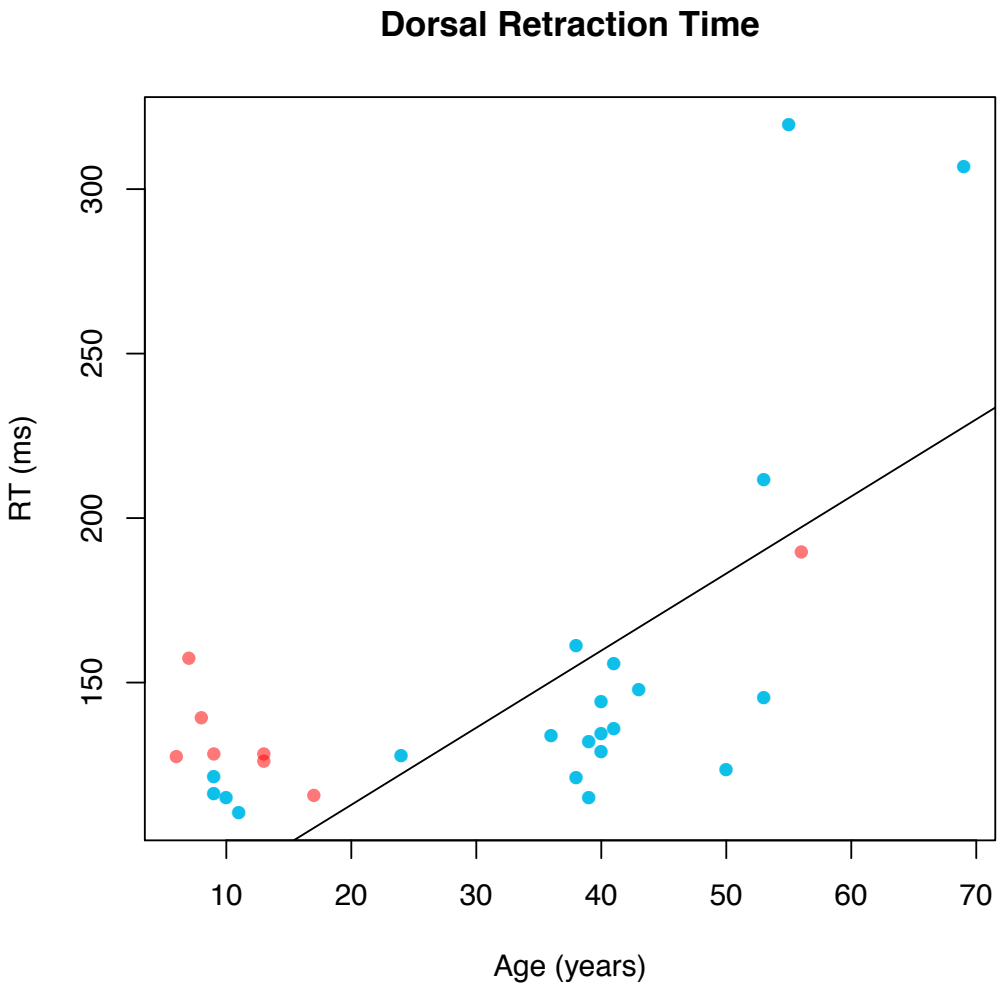

Figure 17. Scatterplot of dorsal retraction time data

Table 12. Ventral RT percent change

	Affected	Unaffected	P-value
Percent Change 1	0.63 ±1.3	1.4 ± 0.78	0.33
Percent Change 2	1.4 ± 1.1	2.2 ± 0.75	0.25
Percent Change 3	1.5 ± 0.8	1.9 ±0.92	0.63
Percent Change 4	1.4 ± 0.8	-0.80 ± 5.0	0.60

Table 13. Dorsal RT percent change

	Affected	Unaffected	P-value
Percent Change 1	1.5 ± 3.2	3.5 ± 2.1	0.30
Percent Change 2	2.7 ± 2.7	4.2 ± 1.6	0.38
Percent Change 3	2.8 ± 2.6	3.8 ± 1.7	0.54
Percent Change 4	2.8 ± 2.5	3.2 ± 1.6	0.80

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