cardiomyocytes with the titin truncating variant showed poorer sarcomere organization compared to wildtype (62.0 ± 3.9 vs. 82.9 ± 2.9; p = 0.008; Figure 1C).

CONCLUSION: Titin truncating variants lead to abnormal sarcomere organization in both atrial and ventricular iPSC-derived cardiomyocytes, and this phenotype can be reverted through CRISPR/Cas9 correction of the titin truncating variant to wildtype. These findings further our understanding of the role of titin in the atria and provide insight to the mechanisms by which titin truncating variants may promote arrhythmogenesis.

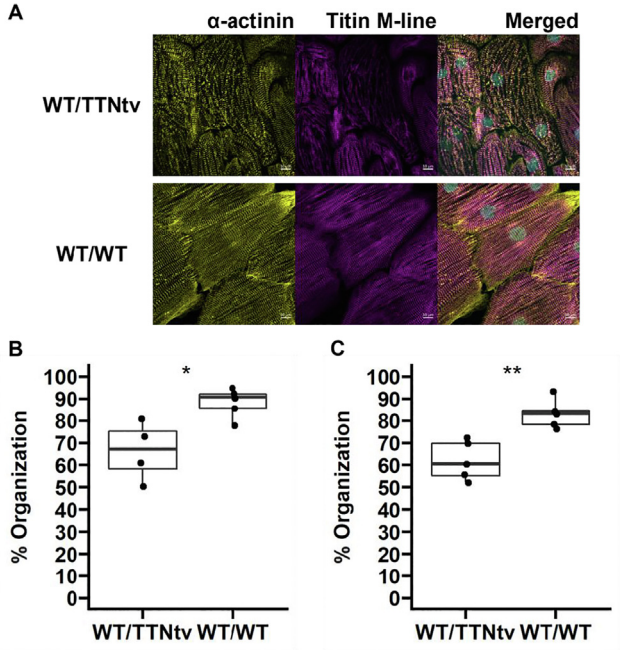

Figure 1. Assessment of sarcomere structures. (A) Confocal microscope images of iPSC-derived atrial cardiomyocytes stained for sarcomere α-actinin, titin, and DNA. Quantification of sarcomere organization in iPSC-derived (B) atrial and (C) ventricular cardiomyocytes (n = 4-5 biological replicates). Abbreviations: TTNtv, titin truncating variant; WT, wildtype.

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P003
CORRELATION OF CELL SENESCENCE WITH THE AGE-ASSOCIATED INCREASE IN VWF EXPRESSION

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BACKGROUND: Von Willebrand factor (VWF) is an endothelial-specific pro-coagulant protein with a major role in thrombosis. It mediates the primary step in thrombogenesis, which is platelet adhesion to the endothelium/sub-endothelium surfaces. Increased VWF level is a significant risk factor for thrombus formation and has been associated with aging. However, the mechanism underlying this age-related increase in VWF remains unknown. We explored the molecular mechanism and functional consequences of age-related upregulation of VWF.

METHODS AND RESULTS: Elisa, western blot and RT-PCR analyses were used to determine circulating plasma levels, cellular protein, and mRNA levels of VWF, in young and aged mice. Immunofluorescent analyses of major organs were performed to establish vascular patterns of VWF and the presence of platelet aggregates. Cultured endothelial cells were used as an in vitro model of aging to explore the mechanism of increased VWF levels. Increased plasma levels of VWF were observed in aged mice. VWF mRNA and protein levels were increased in the endothelium of the brains, lungs and livers, but not kidneys and hearts of aged mice. The distribution of VWF expression in organs was altered from primarily large vessels in young, to include small vessels in the aged mice. Increased platelet aggregates formation in vessels of aged organs was concomitant with increased VWF expression, consistent with increased thrombogenicity. Aspirin treatment significantly reduced platelet aggregates formation in aged mice. Prolonged maintenance of endothelial cells in culture, resulting in cell senescence, correlated with increased VWF at mRNA and protein levels. When cultured endothelial cells were separated into senescent and non-senescent populations VWF levels were consistently higher in the senescent population. Senescence marker β-galactosidase and senescence-associated transcription factor p53 were detected specifically in aged (but not young) brain microvascular endothelial cells that exhibited VWF expression. Aged mice treated with proteolipid vehicles (PLV) encapsulating a DNA-based senolytic targeting senescent cells with elevated p53 transcriptional activity for destruction, exhibited a significant reduction in platelet aggregates formation in the brain vasculatures when compared to control animals.

CONCLUSION: VWF levels and expression patterns were increased in response to aging in an organ-specific manner. This was concomitant with increased platelet aggregate formation, which is a risk factor for age-associated thrombotic disorders. The potential mechanism of age-associated increase in VWF expression may include cell senescence.

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P004
EFFECT OF GLYCOSAMINOGLYCANS ON OPENING ANGLE OF INTACT & CROSSLINKED AORTAS

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BACKGROUND: Ruptures caused by aortic aneurysms and dissections occur when the mechanical stresses in the aortic wall exceed the local aortic strength. In-vivo stresses are strongly mediated by residual stresses (RS), those existing in the absence of

load. Circumferential RS can be estimated ex-vivo via the opening angle (OA), by subjecting an aortic ring to a radial cut. Although the underlying mechanism for RS remains poorly understood, our recent efforts have demonstrated strong correlations between the amounts of sulfated glycosaminoglycans (sGAG) and the OA in healthy aortas. In addition, advanced glycation end products (AGEs) are known to accumulate with age and create crosslinks within the extracellular matrix. Since ruptures are more prevalent in older tissue, the purpose of this study was to compare the effect of sGAG on the OA in intact and glycated aortic tissue.

METHODS AND RESULTS: Sets of four adjacent aortic rings were excised from the upper thoracic regions of 9 porcine aortas. One ring served as a control, while a second ring underwent enzymatic sGAG depletion, a third underwent glycation, and the fourth ring underwent a combination of glycation followed by enzymatic sGAG depletion. A 100mM ammonium acetate buffer, pH 7.0 was used. Glycation and sGAG depletion were induced by incubating samples in 700mM of ribose, and 15U/mL hyaluronidase, 0.075U/mL chondroitinase ABC, 0.75U/mL heparinase for 48 hours at 37oC, respectively. The OA was then measured and the sGAG and general AGE contents were quantified. The quantification of sGAG contents in control and treated samples confirmed the successful removal of sGAG, with reductions by 94±2.5% (average ± standard deviation). In addition, a 93±27% increase in general AGEs was achieved. Statistically significant differences were found between the OAs of control and sGAG depleted, glycated, and glycated combined with sGAG depleted samples (paired sample t-test, $p < 0.001$). Specifically, the OA was reduced by 34±12% after sGAG depletion, 14±8% after glycation, and 45±15% after combined glycation and sGAG depletion. In addition, the OAs in sGAG depleted samples, as well as in combined glycated and sGAG depleted samples, were found to be significantly smaller than samples that underwent glycation only (paired sample t-test, $p=0.002$), being 22±15% and 34±18% smaller respectively.

CONCLUSION: These findings supported that sGAG depletion causes a reduction in the OA, in both intact and glycated tissue, and that AGEs may also affect the magnitude of the RS.

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P006
EICOSAPENTAENOIC ACID (EPA) DECREASES CYTOKINE RELEASE AND EXPRESSION OF INFLAMMATORY AND PRO-THROMBOTIC PROTEINS IN BRAIN VASCULAR ENDOTHELIUM

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BACKGROUND: Brain vascular endothelial cell (EC) dysfunction contributes to ischemic stroke due to inflammation and release of pro-thrombotic factors. Treatment with icosapent ethyl (IPE), the ethyl ester of the omega-3 fatty acid, eicosapentaenoic acid (EPA), reduced first and total ischemic strokes each by 36%, in statin-treated patients with elevated cardiovascular risk (REDUCE-IT). We tested the effects of EPA on cytokine release and expression of inflammatory proteins from brain microvascular ECs during inflammation.

METHODS AND RESULTS: Human brain microvascular endothelial cells were pretreated with the cytokine IL-6 at 12 ng/ml for 2 h before treatment with EPA (40 μM) for 24 h. Proteomic analysis was performed using LC/MS to capture relative expression levels. Only significant changes in protein expression between treatment groups >1-fold were analyzed. Levels of soluble intercellular adhesion molecule-1 (sICAM-1) and tumor necrosis factor-α (TNF-α) were measured by immunochemistry (ELISA). IL-6 exposure produced increased levels of sICAM-1 and TNF-α by 102% and 147% ($p < 0.001$), respectively, in brain ECs compared with vehicle. EPA treatment reduced release of sICAM-1 by 43% ($p < 0.001$) and TNF-α by 52% ($p < 0.001$) compared to IL-6 alone. EPA also decreased expression of 43 proteins involved in the "neutrophil degranulation" pathway in brain ECs (p-adjusted = 2.63×10-12). EPA also decreased expression of prothrombin by 1.3-fold ($p = 2.10 \times 10$-24) relative to IL-6 alone.

CONCLUSION: EPA significantly reduced cytokine release and expression of inflammatory and pro-thrombotic proteins in brain ECs during inflammation. The ability of EPA to reverse brain EC dysfunction and inflammation may contribute to reductions in stroke risk, as demonstrated in large outcome trials.

Amarin Pharma Inc., Elucida Research LLC

Young Investigator Award Winner —Basic Science

P007
ENDOTHELIAL COLONY-FORMING CELL-DERIVED EXTRACELLULAR VESICLES AND CARDIAC REPAIR AFTER MYOCARDIAL INFARCTION

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BACKGROUND: Despite improvements in therapeutics, ischemic heart disease remains a leading cause of death. Cardiac remodeling after myocardial infarction (MI), predominantly due to loss of cardiomyocytes and coronary vasculature, leads to a progressive decline in cardiac function resulting in heart failure. Current therapies for cardiac repair and heart failure are of limited benefit. Cell transplantation therapy upon MI is a very promising therapeutic strategy to replace dead myocardium, reducing scarring and improving cardiac performance.

METHODS AND RESULTS: Our research focuses on endothelial colony-forming cell-derived exosomes (ECFC-exosomes), which are actively secreted endocytic nanovesicles (30-100