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Canadian Cardiovascular Society (CCS) Abstracts — Basic Science

P001
B-MODE ULTRASOUND-GUIDED LOW-LEVEL FOCUSED-ULTRASOUND SONODYNAMIC THERAPY OF NEOINTIMAL HYPERPLASIA USING PHOTOFRIN-LOADED MICROBUBBLES

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BACKGROUND: In vascular surgery, neointimal hyperplasia is a significant clinical phenomenon because it reduces the long-term effectiveness of surgical and endovascular procedures. We devised an experimental sonodynamic therapy protocol and tested its efficacy on neointimal hyperplasia reduction in this study, in which diagnostic B-mode ultrasound is paired with focused-ultrasound with the goal of greater safety.

METHODS AND RESULTS: Golden Syrian hamsters were subjected to endothelial denaturation damage by abdominal aorta ballooning (approximately 0.5 cm superior to the iliac bifurcation). Histopathology results after eight weeks revealed increased smooth muscle cell proliferation in the intimal layer, culminating in vessel wall thickening. The treatment group, then received sonodynamic therapy with pulsed-low-level focused-ultrasound (F= 1.1 MHz, P= 15 W, PD= 250 ms) and sonosensitizer photofrin-loaded PESDA (Perflurocarbon Exposed Sonicated Dextrose Albumin) microbubbles (100ml/kg, 2-5 105 bubbles/ml). The development of inertial cavitation in the abdominal aorta was revealed by B-mode ultrasound imaging combined with ultrasound sonodynamic therapy. In addition, when comparing the treatment group to the other groups, histopathological results revealed a substantial reduction in the mean value for smooth muscle cell density, lumen wall mean thickness, and percentage of luminal cross-sectional area of stenosis ($p < 0.05$).

CONCLUSION: Enhanced cytoxic effect of photofrin, induced by low-level focused-ultrasound sonodynamic therapy combined with enhanced sonoporation effect of focused ultrasound, induced by collapsed bubbles, can cause intimal layer thickness to be reduced and the luminal cross-sectional area of stenosis to be significantly dilated.

Trainee Research Award Finalist — Basic Science

P002
CHARACTERIZING AN ARRHYTHMIA-RELATED TITIN MUTATION USING PATIENT STEM CELL-DERIVED ATRIAL CARDIOMYOCYTES

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BACKGROUND: Atrial fibrillation (AF) is a common arrhythmia that is linked to a greater risk of ischemic stroke and heart failure. Multiple genetic studies have established an association between protein truncating variants in the titin gene and increased risk of AF in the presence or absence of cardiomyopathy. Titin truncating variants are a known cause of dilated cardiomyopathy, thus existing studies have focused on the effects of these variants in ventricular cardiomyocytes. The structural and functional consequences of titin truncating variants in atrial cardiomyocytes and how such variants lead to arrhythmias such as AF are unclear. Our objective was to investigate the cellular effects of titin truncating variants identified in patients with unexplained AF.

METHODS AND RESULTS: We identified a heterozygous titin truncating variant in a patient with early-onset atrial fibrillation and generated induced pluripotent stem cell lines (iPSCs) of the patient. We used CRISPR/Cas9 homology-directed repair to perform genome-editing of the patient iPSCs and corrected the titin truncating variant to wildtype. We differentiated patient iPSCs with the titin truncating variant and wildtype iPSCs into ventricular-like and atrial-like cardiomyocytes. We characterized cellular electrophysiology by optically mapping voltage and calcium transients and assessed the organization of sarcomere structures within cardiomyocytes through immunofluorescent staining of sarcomere proteins and confocal microscopy. iPSC-derived atrial cardiomyocytes displayed cell type-specific characteristics including faster beat rates (mean $\pm$ sem, beats/minute: 150.9 ± 21.8 vs. 21.9 ± 3.6; $p = 0.01$) and shorter rate-corrected action potentials (cAPD80: 195.9 ± 23.8 vs. 394.7 ± 42.8 ms; $p = 0.048$) compared to ventricular cardiomyocytes. Analysis of sarcomere organization showed poorer structural alignment in iPSC-derived atrial cardiomyocytes with the titin truncating variant compared to wildtype (% organization: 66.3 ± 6.8 vs. 88.0 ± 2.9; $p = 0.03$; Figure 1A and 1B). Similarly, iPSC-derived ventricular

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.

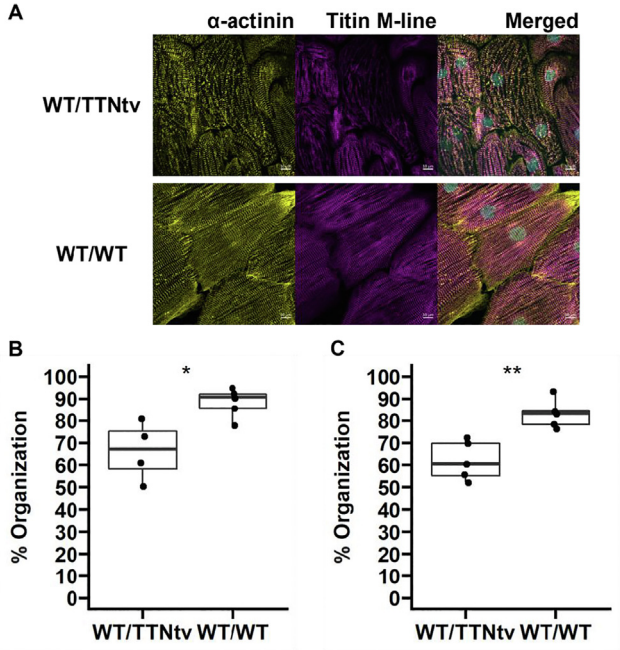

Figure 1. Assessment of sarcomere structures. (A) Confocal microscope images of iPSC-derived atrial cardiomyocytes stained for sarcomere α-actinin, titin, and DNA. Quantification of sarcomere organization in iPSC-derived (B) atrial and (C) ventricular cardiomyocytes (n = 4-5 biological replicates). Abbreviations: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