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In vitro generation of hiPSC-derived megakaryocytes and platelets from a patient with Glanzmann thrombasthenia
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Glanzmann thrombasthenia (GT) is an autosomal recessive platelet disorder characterized by impaired fibrinogen binding due to mutations in the integrin receptor GPIIbIIIa. GT has been studied using animal models, heterologous expression systems and patient-derived platelets. Patient-specific human induced pluripotent stem cells (hiPSC) offer new approaches for modeling blood disorders in vitro by differentiating hiPSC-derived haematopoietic stem cells (HSCs) into myeloid and lymphoid lineages. We have generated patient-specific hiPSC of an 18 year old female carrying two novel compound heterozygous GPIIIa mutations. hiPSC clones were in vitro differentiated into MKs and platelets using a specific protocol. As expected MKs and platelets from both control- and GT-hiPSC showed normal morphology in haemacytomer stains and similar expression of CD42b (GPIb). No surface or intracellular expression of CD41 protein (GPIIa) was detected in MKs and platelets from GT-hiPSC. Upon activation with thrombin, GPIb and A and ADP, peripheral blood-derived and GT-hiPSC-derived platelets reduced PAC1 binding, surface spreading and adherence on fibrinogen, resembling the in vitro phenotype of GT. We demonstrate that GT-hiPSC-derived MKs and platelets recapitulate the disease phenotype in vitro.

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Study of the role of the new endolysosomal receptors two pore channels (TPCN1 and TPCN2) in human, mouse and rat heart
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Purpose: Mammalian two-pore channel proteins (TPCN1, TPCN2) are recently identified as ion channels in intracellular organelles and lysosomes whose role at cardiac level is completely unknown. Our aim was to study the effects of knocking out TPCN1 in mice as well as studying the regulation of expression of cardiac TPCN1 and TPCN2 by cardiovascular diseases and metabolic conditions.
Methods: We carried out a differential proteomic analysis of murine cardiac ventricle biopsies from both TPCN1 knockout (n=3) and control (n=3) mice. Two dimensional gel electrophoresis was performed. Detection was made with SYPRO Ruby and identification by MALDI-TOF/TOF. RT-qPCR was used to quantify mRNA expression and immunoblotting to confirm protein expression of TPCN1 and TPCN2 in left-ventricular myocardium from patients with heart failure (HF, n = 36) without diabetes mellitus of ischaemic (ICM, n = 16) or dilated (DCM, n = 20) cardiomyopathy, and non-diseased donors (CTL, n = 6), as well as in three four-week-old male Sprague-Dawley rats fed with standard or high-fat diet in male and female Zucker diabetic fatty (ZDF) and lean (ZL) rats.
Results: In murine cardiac tissue, we observed 27 differential spots (fold change: 1.8 and p<0.05), all elevated in TPCN1 KO versus wt mice. The most relevant protein change observed was FABP3 (Fatty acid binding protein 3) and it was validated by western blot (p=0.039).
Cardiac transcript expression of TPCN1 (p=0.018) and TPCN2 (p=0.001) increased in heart failure patients relative to controls. Increases remained significant for TPCN2 in all groups (ICM, p=0.008; DCM, p=0.001) but for TPCN1 only in DCM (p=0.015).
In male Sprague-Dawley rats, we observed an increase in TPCN1 (p=0.012) and TPCN2 (p=0.0041) cardiac mRNA levels in high fat (n=13) versus control (n=13) rats. On the contrary, in Zucker rats, we observed a decrease of TPCN1 (p=0.001) and TPCN2 (p=0.0005) mRNA levels in ZL (n=10) versus ZDF (n=10).
Conclusion: In TPCN1 KO mice heart, there is an increase in FABP3 which suggest that cardiac fatty acid transport is altered by the lack of TPCN1. Both receptors seem to be key regulators in the rat heart metabolism, since their gene expression levels are regulated by diet and obesity. TPCN1 expression is clearly increased in HF as a whole also tended towards a cardio-metabolic difference. TPCN2 regulation should also be involved in a general mechanism underlying heart failure, at least with ICM and DCM etiology. The current data provide the first evidence that the endolysosomal system might be involved in the regulation of cardiac metabolism.

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In situ generation of hiPSC-derived megakaryocytes and platelets from a patient with Glanzmann thrombasthenia
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Glucose depletion is essential for the calorie-restriction-mediated cardioprotective hypertrophic signalling pathways TPCN1
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Purpose: In last ESC meeting, we presented the impact of calorie restriction (CR) on cardiac remodeling observed in diet-induced obesity mice and pressure over-load heart failure mouse model (ESC 2012, YIA Runner of basic research); however, it remains unclear whether the essential nutrient factor(s) that may be responsible for the CR-mediated enhanced cardiac angiogenesis and M2 the underlying molecular mechanism(s).
Methods: Using both murine models of heart failure and cultured cardiomyocytes and endothelial cells, we examined mechanisms of CR-induced cardiac angiogenesis. Eighteen-week-old diet-induced obesity (DIO) mice, as a chronic heart