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Cardiomyocytes derived from induced pluripotent stem cells of patient with DiGeorge syndrome show slower beating frequency and higher irregularity

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Human induced pluripotent stem cells (hiPSCs) and their differentiated derivatives are new, promising models for studying disease-related phenotypes in vitro. DiGeorge syndrome is caused by the deletion of the 22q11.2 chromosome region on one allele and the hemizygous presence of the affected genes is not sufficient for the healthy phenotype. DGCR8 gene encoding a key component of the microprocessor complex essential for microRNA biogenesis, located in this region. We generated iPSCs from peripheral blood mononuclear cells of members of a family where the disease is present in three generations. The manifestations of the disease differ between family members as grandfather and mother have milder symptoms including minimal facial dysmorphia and hypocalcaemia, while progeny had severe symptoms including pulmonary atresia, ventricular and atrial septal defect and hypoparathyroidism. MLPA genetic assay shows that mother and progeny lack the same 3 Mb long chromosome region and karyotype analysis showed normal chromosomal arrangement in both cases. The unaffected father has no microdeletion but karyotype analysis revealed a translocation between chromosomes 6 and 12. Reprogramming of cells was performed by the expression of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) via Sendai virus vector transduction. Generated iPSCs were characterised by mRNA and protein expressions of pluripotency markers Nanog (ΔCt father: 0.034, mother: 0.069, progeny: 0.049, $p>0.05$) and Oct4 (ΔCt father: 0.48, mother: 0.50, progeny: 0.39, $p>0.05$). FACS revealed >90% of the cells were positive for SSEA4 cell surface pluripotency marker. mRNA levels of DGCR8 were decreased to 41% in mother ($n=3$, $p<0.05$) and to 26% in progeny ($n=3$, $p<0.01$) compared to father reflecting the genetic background. The hiPSCs were differentiated into endothelial cells and cardiomyocytes to compare phenotypes of disease-affected and control cells. During endothelial differentiation, expression of mesodermal markers Mesp1 and Brachyury was increased at day5 ($n=3$, $p<0.0001$) and CD31 ($n=3$, $p<0.05$). VE-cadherin and Angiopoietin2 ($n=3$, $p<0.0001$) endothelial markers showed increased expression on day 19. CD31/NRP1-double positive arterial like endothelial cells were characterized by immunocytochemistry, tube formation and ac-LDL assays. Beating cardiac clusters showed significantly decreased frequency (control: 76.3±0.9/min, progeny: 57.7±4.9/min, $p<0.0001$) and higher beating rhythm irregularity index (control: 0.03±0.04, progeny: 0.09±0.12, $p<0.5$) in case of progeny ($n=108$) compared to healthy hiPSC line XCL1 ($n=10$) on day 18 of cardiac differentiation. Our study can provide insights to the development of disease and may serve as human in vitro model for design of new drug targets in complex multiorgan disorder. Further comparative analyses on cellular morphology, viability, transcriptomics, proteomics and functionality are warranted.