P19 Low levels of the A3243G MTDNA mutation in human induced pluripotent stem cell-cardiomyocytes do not cause functional or metabolic disturbances but increase with further passaging

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The heteroplasmic mtDNA mutation A3243G can cause the mitochondrial condition MELAS. Mitochondrial replacement therapy can prevent transmission of mtDNA mutations to offspring but to maintain nuclear integrity, a certain amount of cytoplasm and mutated mtDNA is carried over (<3%). It is unknown whether this will increase with age and this is particularly relevant in the heart, where mutations accumulate over time. We applied small molecule modulation of the Wnt/β-catenin signalling pathway to generate pure populations of cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) from a patient with 20% heteroplasmy for the A3243G mtDNA mutation No changes in the basal beating rate or time to peak and time to 50% relaxation were found. No differences in the response to β-adrenergic stimulation by isoprenaline or muscarinic inhibition by carbachol. A3243G hiPSC-CMs showed reduced excitability (18.85±3.045 ms for control and 38.08±6.126 ms for A3243G, Mean ±SEM, p=0.0084) but there were no changes in other calcium handling properties. Mitochondrial DNA copy number and both mitochondrial respiration and basal glycolysis were unaffected. We have seen a gradual increase in A3243G hiPSCs and derived CMs heteroplasmy with passaging (26.4% to 38.7% over 6 passages). We conclude that A3243G heteroplasmy <40% is not sufficient to affect the generation of hiPSC-CMs and their beating, calcium handling and metabolic properties. Having observed an increase in heteroplasmy with passaging, these results provide useful insights into changes that might happen with age in children resulting from mitochondrial replacement therapy.