Sphingosine-1-Phosphate Lyase (*SGPL1*) Deficiency is Associated with Mitochondrial Dysfunction

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Introduction: Loss of function mutations in *SGPL1*, a key component of sphingolipid metabolism, are associated with accumulation of sphingolipid intermediates giving rise to a multi-systemic disease incorporating primary adrenal insufficiency (PAI) and progressive renal and neurological disease. Sphingolipids are implicated in mitochondrial apoptosis via induction of mitochondrial outer membrane permeabilization, cytosolic release of inter-membranal cytochrome c and activation of executioner caspases.

Background: Several sphingolipid intermediates such as ceramide, sphingosine and sphingosine 1-phosphate have been shown to act as modulators of the steroidogenic pathway often acting as second messengers altering downstream expression of steroid responsive transcriptional elements.

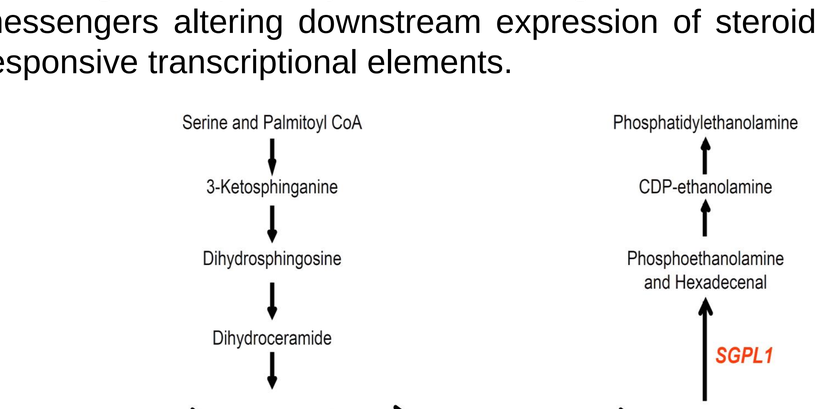


Figure 1: SGPL1 carries out final degradative step in sphingolipid metabolism.

Figure 3: Total mitochondrial volume in patient fibroblasts and SGPL1-KO-HeLa cell lines vs controls was reduced (P<0.05): Additionally, the number of fragmented mitochondria was increased in p.S65Rfs*6G compared to control (P<0.0001).

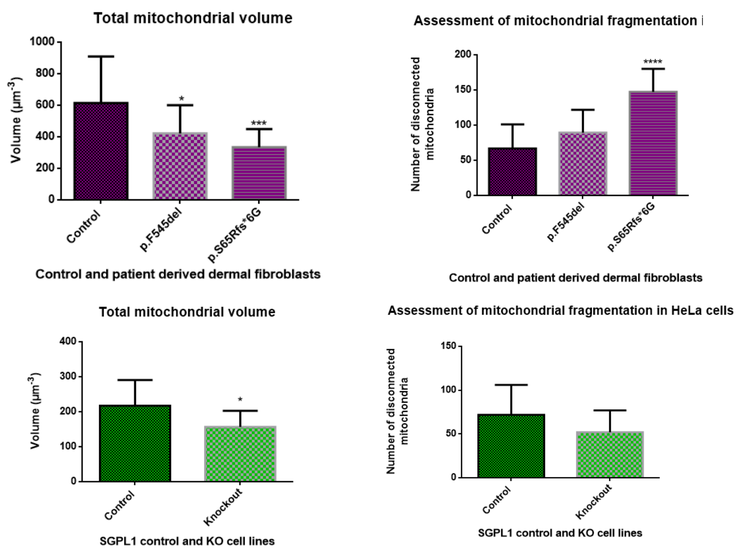
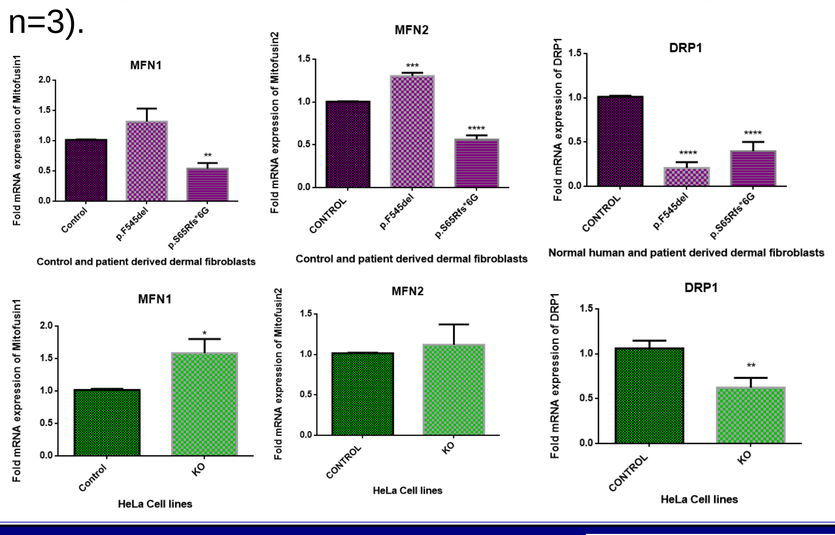


Figure 4: *MFN1* and *MFN2* expression were markedly upregulated in patient 1 p.F545del fibroblasts (p<0.0001; n=3) while the opposite was seen in p.S65Rfs*6G (p<0.0001; n=3). However, *DRP1* was uniformly downregulated in both patient fibroblasts (p<0.0001, n=3).



Objectives and hypothesis: To investigate the impact of SGPL1 deficiency on mitochondrial morphology and function using patient derived human dermal fibroblasts and a SGPL1- knockout HeLa cell line.

Methods:

Primary cell cultures of dermal fibroblasts were established from two patients with SGPL1 deficiency (Patient 1 - p.F545del; PAI, later onset renal/neurological compromise; Patient 2 - renal/neurological compromise). Mitochondrial architecture was examined by confocal microscopy with volumetric analysis using Z-stack images of stained cells. Mitochondrial oxidative phosphorylation rate was measured by Seahorse XF Extracellular Flux Analyser in control/patient fibroblasts. RT-qPCR for expression levels of genes regulating mitochondrial fusion and fission, *MFN1/2* and *DRP1*

Results: ***Mitochondrial morphology differed; SGPL1-KO-HeLa and p.F545del had elongated and hyper-fused mitochondria whereas p.S65Rfs*6G had rounded, fragmented mitochondria. (Figure 2)***

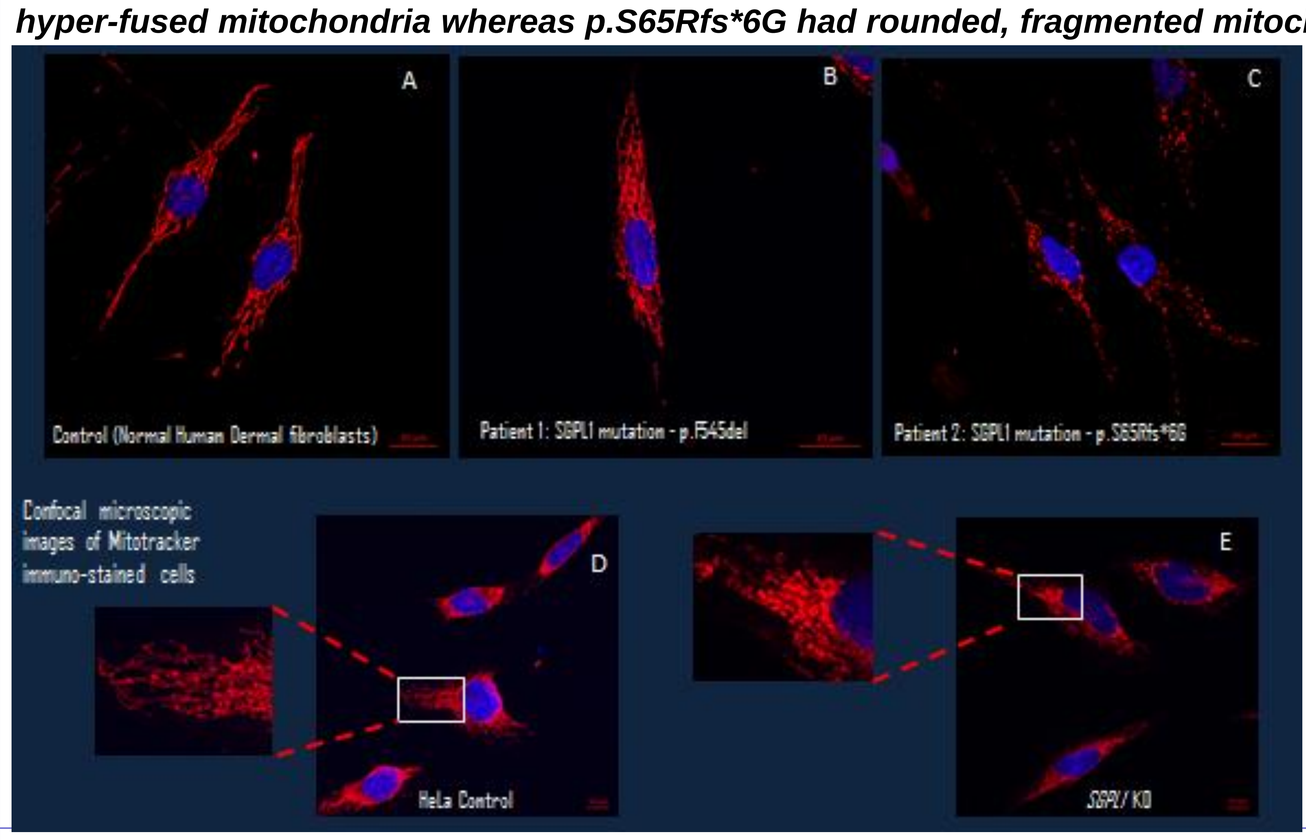
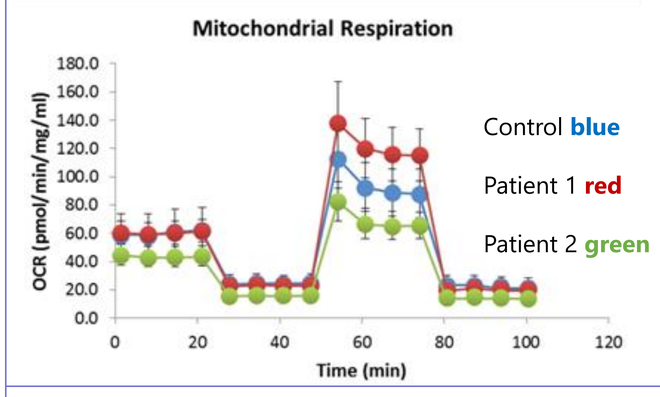


Figure 2: Confocal microscopic images of Mitotracker immuno-stained cells.

Panels A-C show altered mitochondrial phenotypes between control and patient fibroblasts.

Panels D and E show HeLa control and *SGPL1* knockout cells respectively.

Figure 5: *Oxygen consumption rates (OCR) of control and patient fibroblasts following injection of optimal concentrations of Oligomycin (4 µM), FCCP (1 µM) and Rotenone/antimycin A (1 µM), indicated by numbers 1-3 respectively*



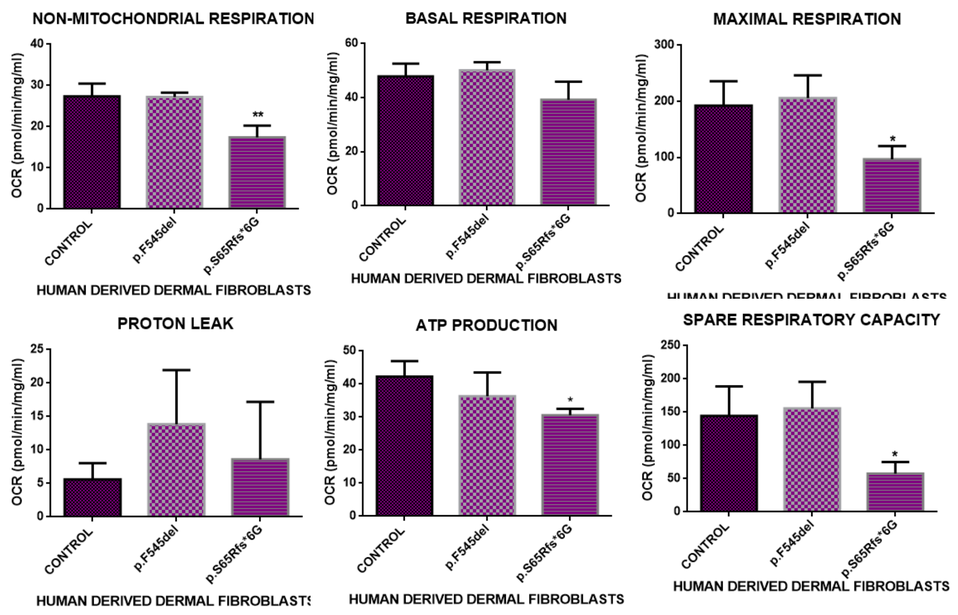


Figure 6: The respiratory flux profile of p.F545del fibroblasts was unaltered, however, p.S65Rfs*6G fibroblasts showed a significant reduction in non-mitochondrial respiration (P<0.01, n=3, maximal respiration (P<0.05), ATP production (P<0.05) and spare respiratory capacity.

Conclusion: Aberrant sphingolipid metabolism leads to disruption of mitochondrial morphology/function. The significant decreased DRP1 expression suggests an imbalance tilted towards reduced fission. The degree of SGPL1 deficiency or other genetic modifiers may account for differences seen.

However, importantly, in both patient fibroblasts and SGPL1-KO-HeLa cells mitochondrial volume is reduced. Further work is required to characterise mitochondrial effects of SGPL1 deficiency.

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Adrenals and HPA Axis

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