

Table of Contents

1	Introduction and background	7
1.1	Inborn errors of metabolism	7
1.1.1	Mitochondrial disorders	7
1.1.2	Glycogen storage disease	11
1.2	Aim of this work	13
2	Material & Methods	14
2.1	Patients	14
2.2	Materials	14
2.3	Whole-exome sequencing	14
2.4	Cloning	15
2.5	Chemically competent cells	17
2.6	Transformation of competent cells	21
2.7	TELT-Prep	21
2.8	Cells and cell culture	22
2.9	Generation of knockout cell lines by CRISPR/Cas9	22
2.10	Immortalization of B lymphocytes	23
2.11	Transfection	23
2.12	Immunofluorescence staining	23
2.13	Microscopy	23
2.14	Yeast-two-hybrid	24
2.15	Immunoprecipitation	24
2.16	Protein extracts	25
2.17	SDS-gel electrophoresis, Western blot and immunodetection	25
2.18	Histology	27
2.19	Electron Microscopy	27
2.20	Flow cytometry	27
2.20.1	Mitochondrial Membrane Potential	27

2.20.2	Reactive Oxygen Species	28
2.20.3	Cell Cycle	28
2.20.4	Apoptosis	29
2.21	Biochemical examination OXPHOS function	29
2.22	Growth Curve	29
2.23	Quantitative polymerase chain reaction (qPCR).....	29
2.24	Quantitative reverse transcription PCR (RT-qPCR).....	29
2.25	Blocking in cell cycle phases.....	30
2.26	RNA-Sequencing.....	31
2.27	Statistics.....	31
3	Results	32
3.1	Clinical description and genetic analysis of the index patient	32
3.2	Subcellular localization	46
3.3	Yeast-two-hybrid screen and RNA-Seq	50
3.4	Cell Cycle and Apoptosis	54
3.5	Mitochondrial Function	58
4	Discussion	64
4.1	C2orf69 in mitochondrial function	65
4.2	C2orf69 in inflammation	69
4.3	C2orf69 in cell cycle regulation.....	70
4.4	C2orf69 and GSD type IV	71
5	Conclusion.....	72
6	References	75
7	Supplement.....	80
8	Eidesstattliche Erklärung	86