**Genomic and metabolic evaluation of the HEK 293 cell line for use in genetic disease modelling of inherited fatty acid ß-oxidation disorders**

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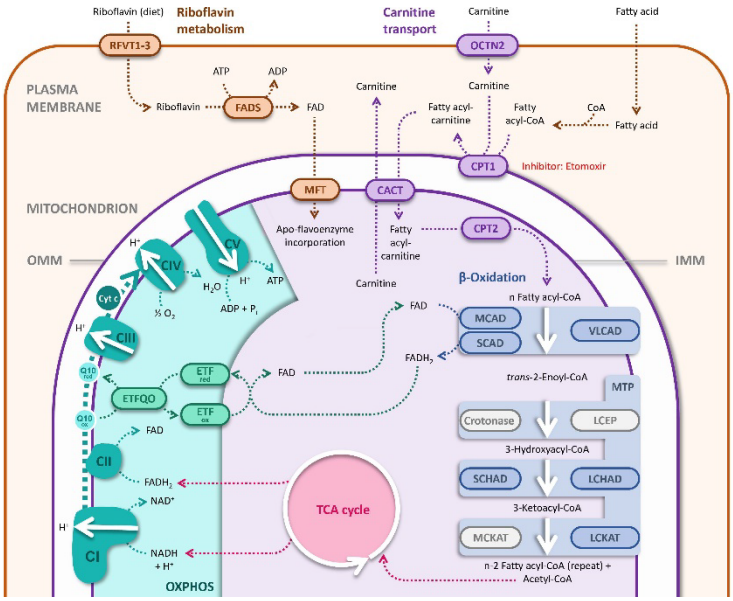


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| ENZYME | GENE | DISORDER | CHR. POS. | HEK 293 CELL LINE* |
|---|---|---|---|---|
| **PRIMARY FAODs** | | | | |
| OCTN2 | *SLC22A5* | OCTN2 deficiency | 5q31.1 | Unaffected |
| CPT1A | *CPT1A* | CPT1A deficiency | 11q13.3 | Unaffected |
| CACT | *SLC25A20* | CACT deficiency | 3p21.31 | Unaffected |
| CPT2 | *CPT2* | CPT2 deficiency | 1p32.3 | Unaffected but variable |
| VLCAD | *ACADVL* | VLCAD deficiency | 17p13.1 | Unaffected |
| MTP | *HADHA & HADHB* | MTP deficiency | 2p23.3 | Unaffected |
| MTPα | *HADHA* | LCHAD deficiency | 2p23.3 | Unaffected |
| MTPβ | *HADHB* | LCKAT deficiency | 2p23.3 | Unaffected |
| MCAD | *ACADM* | MCAD deficiency | 1p31.1 | Unaffected but variable |
| SCAD | *ACADS* | SCAD deficiency | 12q24.31 | Unaffected |
| SCHAD | *HADH* | SCHAD deficiency | 4q25 | Unaffected |
| **SECONDARY FAODs** | | | | |
| RFVT1 | *SLC52A1* | RFVT deficiency | 17p13.2 | Unaffected but variable |
| RFVT2 | *SLC52A2* | RFVT deficiency | 8q24.3 | Unaffected |
| RFVT3 | *SLC52A3* | RFVT deficiency | 20p13 | Unaffected |
| FADS | *FLAD1* | FADS deficiency | 1q21.3 | Unaffected but variable |
| MFT | *SLC25A32* | MFT deficiency | 8q22.3 | Unaffected |
| ETFQO | *ETFDH* | MADD/GA2 | 4q32.1 | Unaffected |
| ETFα | *ETFA* | MADD/GA2 | 15q24.2-q24.3 | Unaffected but variable |
| ETFβ | *ETFB* | MADD/GA2 | 19q13.41 | Unaffected |

*Based on data available*

**Graphic Summary | Overview of the enzymes and genes involved in mitochondrial fatty acid β-oxidation (FAO) and its associated genetic disorders**

**Introduction**: Mitochondrial fatty acid β-oxidation (FAO) is central to the production of cellular energy, especially during the postabsorptive and fasted states. Its importance is exemplified by the existence of 16 inborn errors of metabolism (IEM) caused by pathogenic variants in at least 19 genes. Indeed, FAO disorders (FAODs) are among the most frequently diagnosed IEM in the genetically distinct South African population, are clinically heterogeneous, and therefore exhibit much difficulty regarding their diagnosis and successful treatment. Consequently, there is a growing demand for disease models in which novel, population-specific variants can be investigated. To this end, the HEK 293 cell line is extensively used in biomedical research fields. Since immortalised cell cultures are, however, known to exhibit varying numerical and structural chromosomal anomalies, it is imperative to characterise the HEK 293 cell line on a genomic and metabolic level prior to implementing it as a disease model.

**Methodology**: In this study, HEK 293 cells were karyotyped via G-banding to determine the chromosomal copy number and any possible aberrations. Whole exome sequencing (WES) was performed to identify variants present in the 19 candidate genes, after which the pathogenicity of each variant was evaluated via online databases and the ACMG (American College of Medical Genetics and Genomics) guidelines. To determine the metabolic suitability of HEK 293 as a basis for disease modelling of FAODs, acyl-carnitine levels were quantified in untreated and CPTI-inhibited (etomoxir) HEK 293 cells, using LC-QQQ and stable isotopes.

**Results**: G-banding analysis confirmed the HEK 293 cell line to be near-triploid with a modal number of 66 (60 to 69, n=22) and revealed multiple numerical and structural chromosomal anomalies, as predicted. By comparison to the human reference genome (GRCh37.p13), WES of HEK 293 further yielded a total of 40 benign variants (13 intron-, 1 splice-, 6 upstream-, 2 downstream-, 11 synonymous- and 7 missense variants) in *SLC22A5, CPT1A, SLC25A20, CPT2, HADHA, HADHB, ACADM, ACADS, HADH, SLC52A1-3, SLC25A32, ETFDH, ETFA,* and *ETFB*. Mass spectrometry analysis of the acyl-carnitine profile also corroborated the correct functioning of mitochondrial FAO in the HEK 293 cell line, in the absence and presence of etomoxir-mediated CPT1 inhibition.

**Discussion and Conclusion**: While none of the chromosomal aberrations noted are predicted to directly affect the disease genes of interest, the generation of genetic disease models in which the genes are located on chromosomes 1 [MCAD- (*ACADM*), CPT2- (*CPT2*), and FADS *(FLAD1*) deficiency], 15 [MADD/GA2 (*EFTA*)], and 17 [VLCAD- (*ACADVL*) and riboflavin (*SLC52A1*) deficiency] should be approached with caution, since these chromosomes exhibit great variation regarding chromosomal number and structural changes. Concerning the genetic suitability of FAO genes, HEK 293 cells represent a healthy wildtype basis in which genetic alterations can be induced via highly specific methods, such as CRISPR/Cas, to successfully produce population-specific FAOD models. Finally, HEK 293 cells exhibit a functional mitochondrial FAO pathway which reacts as predicted when challenged chemically. Consequently, this study does not reveal any major concerns in terms of the karyotype, genetic variants, and mitochondrial FAO of the HEK 293 cell line, thereby encouraging its use for the generation of genetic models to better study and comprehend FAODs.

**Keywords:** Fatty acid β-oxidation disorders, whole exome sequencing, G-banding, HEK 293, CRISPR/Cas, disease model