**SUPPLEMENTARY INFORMATION**

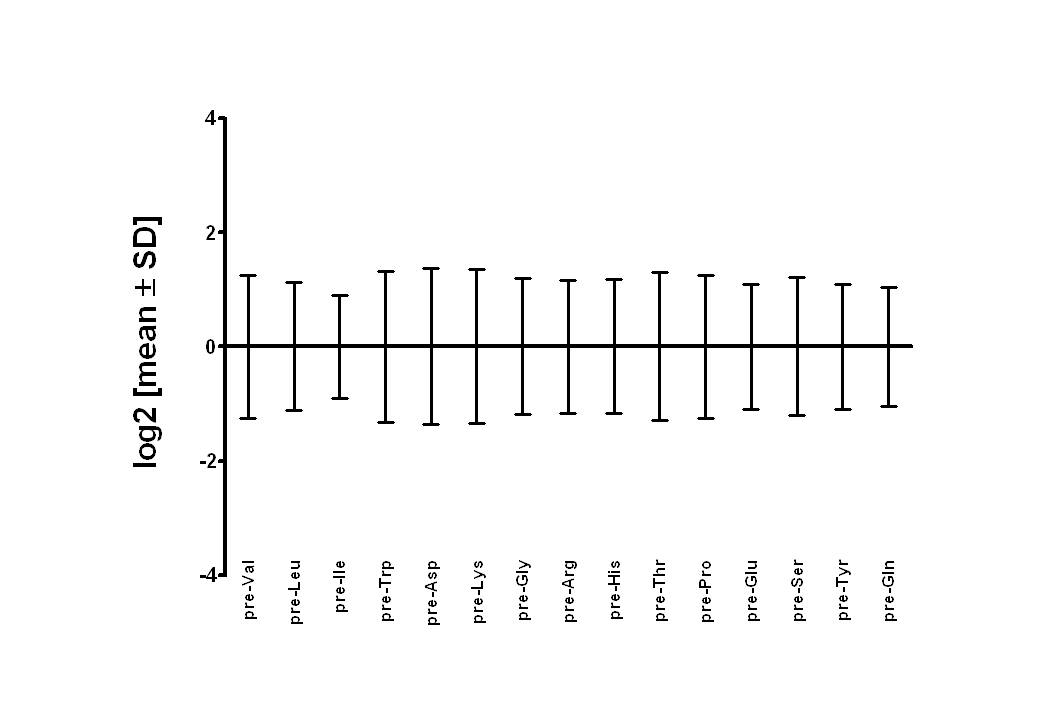
**SUPPLEMENTARY FIGURES**

**Fig S1ABC fertig 03_02_2014 komprimiert.tif**

**Figure S1** qRT-PCR analysis of RNase P components(A)Analysis of different siRNAs targeting *HSD17B10* in HEK-293 cells. siRNA 2 yielded in a knock-down of *HSD17B10* mRNA of approximately 90%. (B) siRNAs targeting MRPP1 were tested using qRT-PCR analysis. (C) mRNA levels of RNase P constituentswere measured in control and patient fibroblasts. HSD10 mutations did not alter transcript levels of either *HSD17B10*, *RG9MTDI* nor *KIAA0391*.

Supplemental Fig 2.tif

**Figure S2** Effects of p.R130C mutation on recombinant expression and structural integrity   
(A) Coomassie staining of SDS-PAGE following small-scale *E. coli* expression and affinity purification of human HSD10 wt and mutants. In short, cells expressing recombinant HSD10 were grown in 50 ml TB medium, induced with 0.1 mM IPTG at 18°C overnight, harvested by centrifugation at 4000 xg, lysed by sonication and purified by affinity chromatography (Ni-NTA; Qiagen) . For each protein, lanes for total cell lysate ('T') and eluat after nickel purification ('E') are shown. (B) Structural environment of the p.R130C mutation site (red stick) in the wt structure (PDB code 2O23). A neighbouring subunit in the HSD10 tetramer (grey ribbon) is shown. Hydrogen bonds are indicated in dotted lines (distances in angstrom).

****

**Figure S3** Calculation of the standard deviation of control fibroblasts. qPCR analysis was performed for all control fibroblasts and their standard deviation was calculated. Based on the microarray analysis a three times higher signal than the background was consider as significant signal and used as a threshold for the accumulation of tRNA precursors.

Fig S4 A,B,C final version.tif

**Figure S4** (A) Schematic representation of the mitochondrial heavy and light strand with genes ordered according to their position in the mitochondrial genome. Position of interspersed tRNAs are indicated as horizontal black bars. (B) Northern blots were hybridized with specific probes for tRNAGln, tRNASer(UCN), and 12S rRNA. These specific probes gave a signal, which corresponds to their respective tRNAs; no precursor transcripts were found except for 12S rRNA indicating an higher molecular weight RNA species corresponding to RNA 1.1 described in Fig 5B. In further studies, northern blots were analysed with a specific probe for 18S rRNA and subsequently with a specific probe for MT-CO2. Specific signals were found for MT-CO2 transcripts (709bp) and nuclear coded 18S rRNA (1.9kb). Additionally, two precursor transcripts were found in fibroblasts from HSD10 patients which correspond to RNA 2.1 and RNA 2.2, respectively, as shown in figure 5B and described in the result section.

.

**SUPPLEMENTARY TABLES**

**Table S1** Genetic, Biochemical and Clinicial Characterization of patients with HSD10 disease.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Patient | Sex | Mutation  (cDNA) | Functional Effect | Manifestation form | MHBD activity  (nmol/min x mg) | Controls  (mean ±SD) | Referenz |
| 1 | M | c.388C>T | p.R130C | infantile | 0.02/ 0.39 ± 0.19\* | 1.48 ± 0.12 | Zschocke et al. 2000 (1) |
| 2 | F | c.388C>T | p.R130C | non-progressive ID | 1.01 ± 0.05 | 7.1 ± 0.83 | Ensenauer et al. 2002 (2) |
| 3 | M | c.388C>T | p.R130C | infantile | 0.57 ± 0.17 | 7.1 ± 0.83 | Ensenauer et al. 2002 (2) |
| 4 | M | c.745G>C | p.E249Q | juvenile | 0.89 | 7.3 ± 0.7 | Olpin et al. 2002 (3) |
| 5 | M | c.388C>T | p.R130C | infantile | not detectable | 7.27 ± 1.16 | Sutton et al. 2003(4) |
| 6 | M | c.388C>T | p.R130C | infantile | not detectable | 1.06 ± 0.54 | Sass et al. 2004 (5) |
| 7 | M | c.364C>G | p.L122V | infantile | 0.7 | 7.1 ± 0.8 | Poll-The et al. 2004 (6) |
| 8(a) | F | c.740A>G | p.N247S | psychomotor delay,  hearing loss | 3.81 | 7.27 ± 1.16 | Perez-Cerda et al. 2005 (7) |
| 9(a) | M | c.740A>G | p.N247S | neonatal | 1.5 | 7.27 ± 1.16 | Perez-Cerda et al. 2005 (7) |
| 10 | M | c.388C>T | p.R130C | early infantile | 0.8 | 7.27 ± 1.16 | García-Villoria et al. 2005 (7) |
| 11 | M | c.388C>T | p.R130C | infantile | not specified | - | Cazorla et al. 2007 (8) |
| 12 | M | c.628C>T | p.P210S | early infantile | non detectable | 1.4 ± 0.43 | García-Villoria et al. 2009 (9) |
| 14 | M | c.388C>T | p.R130C | infantile | 0.05 | 1.4 ± 0.43 | García-Villoria et al. 2009 (9) |
| 15 | M | c.667G>A | p.R226Q | neonatal | 0.27 | 1.4 ± 0.43 | García-Villoria et al. 2009 (9) |
| 16 | M | c.628C>T | p.P210S | infantile | 0.5 | 1.4 ± 0.43 | García-Villoria et al. 2009 (9) |
| 17(1) | M | c.257A>G | p.D86G | neonatal | 0.72/ 2.8 | 1.04 ± 0.5/ 7.3 ± 1.2 | Rauschenberger et al. 2010 (10) |
| 18(b) | M | c.495A>C | p.Q165H | microcephaly, mild growth retardation | 0.2 | 7.3 ± 1.2 | Rauschenberger et al. 2010 (10) |
| 19(b) | M | c.495A>C | p.Q165H | microcephaly, mild growth retardation | 0.2 | 7.3 ± 1.2 | Rauschenberger et al. 2010 (10) |
| 20(c) | M | c.495A>C | p.Q165H | asyptomatic | 0.2 | 7.3 ± 1.2 | Rauschenberger et al. 2010 (10) |
| 21 | M | c.388C>T | p.R130C | infatile | Not specified | - | Zschocke et al. 2012 (11) |
| 22 | M | c.388C>T | p.R130C | late infantile | Not specified | - | Zschocke et al. 2012 (11) |

\*MHBD activity in the fibroblasts of the index patient after a further measurement by the group of Zschocke et al.

(a) (b)These individuals are siblings. (c) Cousin of patients 19, 20.

(1)MHBD activity in patient 18 was measured in two independent laboratories using different assays.

Reference List

1 Zschocke, J., Ruiter, J.P., Brand, J., Lindner, M., Hoffmann, G.F., Wanders, R.J. and Mayatepek, E. (2000) Progressive infantile neurodegeneration caused by 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency: a novel inborn error of branched-chain fatty acid and isoleucine metabolism. *Pediatr. Res.*, **48**, 852-855.

2 Ensenauer, R., Niederhoff, H., Ruiter, J.P., Wanders, R.J., Schwab, K.O., Brandis, M. and Lehnert, W. (2002) Clinical variability in 3-hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency. *Ann. Neurol.*, **51**, 656-659.

3 Olpin, S.E., Pollitt, R.J., McMenamin, J., Manning, N.J., Besley, G., Ruiter, J.P. and Wanders, R.J. (2002) 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency in a 23-year-old man. *J. Inherit. Metab. Dis.*, **25**, 477-482.

4 Sutton, V.R., O'Brien, W.E., Clark, G.D., Kim, J. and Wanders, R.J. (2003) 3-Hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency. *J. Inherit. Metab. Dis.*, **26**, 69-71.

5 Sass, J.O., Forstner, R. and Sperl, W. (2004) 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency: impaired catabolism of isoleucine presenting as neurodegenerative disease. *Brain Dev.*, **26**, 12-14.

6 Poll-The, B.T., Wanders, R.J., Ruiter, J.P., Ofman, R., Majoie, C.B., Barth, P.G. and Duran, M. (2004) Spastic diplegia and periventricular white matter abnormalities in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, a defect of isoleucine metabolism: differential diagnosis with hypoxic-ischemic brain diseases. *Mol. Genet. Metab.*, **81**, 295-299.

7 Perez-Cerda, C., Garcia-Villoria, J., Ofman, R., Sala, P.R., Merinero, B., Ramos, J., Garcia-Silva, M.T., Beseler, B., Dalmau, J., Wanders, R.J. *et al.* (2005) 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency: an X-linked inborn error of isoleucine metabolism that may mimic a mitochondrial disease. *Pediatr. Res.*, **58**, 488-491.

8 Cazorla, M.R., Verdú, A., Pérez-Cerdá, C. and Ribes, A. (2007) Neuroimage Findings in 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency. *Pediatr. Neurol.*, **36**, 264-267.

9 Garcia-Villoria, J., Navarro-Sastre, A., Fons, C., Perez-Cerda, C., Baldellou, A., Fuentes-Castello, M.A., Gonzalez, I., Hernandez-Gonzalez, A., Fernandez, C., Campistol, J. *et al.* (2009) Study of patients and carriers with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency: difficulties in the diagnosis. *Clin. Biochem.*, **42**, 27-33.

10 Rauschenberger, K., Scholer, K., Sass, J.O., Sauer, S., Djuric, Z., Rumig, C., Wolf, N.I., Okun, J.G., Kolker, S., Schwarz, H. *et al.* (2010) A non-enzymatic function of 17beta-hydroxysteroid dehydrogenase type 10 is required for mitochondrial integrity and cell survival. *EMBO Mol. Med.*, **2**, 51-62.

11 Zschocke, J. (2012) HSD10 disease: clinical consequences of mutations in the HSD17B10 gene. *J. Inherit. Metab. Dis.*, **35**, 81-89.

**Table S2** Primer sequences for qRT-PCR

|  |  |
| --- | --- |
| **Primer** | **sequence 5’ to 3’** |
| HSD17B10 fwd  HSD17B10 rev | CCCAAGCCAAGAAGTTAGGA  CACAGTTGACAGCTACATCC |
| RG9MTD1 fwd  RG9MTD1 rev | CACTATCTCTGCACTCCTGG  CAAAAACCTGGTGAAAGGTCT |
| KIAA0391 fwd  KIAA0391 rev | AATGGAAAGGACAATTCACC  TAAGACATTCATATTCTTCTGGAC |
| GAPDH fwd  GADPH rev | GTGTGAACCATGAGAAGTATGAC  TTCCACGATACCAAAGTTGTC |

**Table S3** Sequences used for qPCR of unprocessed mt-tRNA transcripts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Nr.** | **Target** | **Or.** | **sequence 5’ to 3’** | **primer-length** | **product-length** | **mitochondrial position of amplified sequence** |
| 1 | pre Val | fwd | CTAAAACCCCTACGCATTTATATAGAG | 27 | 127 | 1525-1651 |
|  |  | rev | TTGAAATCTCCTAAGTGTAAGTTGGG | 26 |  |  |
| 2 | pre Leu | fwd | ATATCATCTCAACTTAGTATTATACCC | 27 | 100 | 3180-3279 |
|  |  | rev | CTGTAAAGTTTTAAGTTTTATGCGA | 25 |  |  |
| 3 | pre Ile | fwd | ATATGATATGTCTCCATACCCATTAC | 26 | 111 | 4210-4320 |
|  |  | rev | GGGTTTAAGCTCCTATTATTTACT | 24 |  |  |
| 4 | pre Met | fwd | TAGGACTATGAGAATCGAACCC | 21 | 133 | 4330-4463 |
|  |  | rev | GGGAAGGGTATAACCAACATT | 21 |  |  |
| 5 | pre Trp | fwd | CCTTACCACGCTACTCCTAC | 20 | 102 | 5462-5563 |
|  |  | rev | CAACTTACTGAGGGCTTTGAA | 21 |  |  |
| 6 | pre Asp | fwd | AAACCTGGAGTGACTATATGGA | 22 | 119 | 7373-7491 |
|  |  | rev | GTTGGCTTGAAACCAGCTTT | 20 |  |  |
| 7 | pre Lys | fwd | AATTCCCCTAAAAATCTTTGAAATAGG | 27 | 123 | 8224-8346 |
|  |  | rev | GGTTCTCTTAATCTTTAACTTAAAAGG | 27 |  |  |
| 8 | pre Gly | fwd | CTTCATCCGCCAACTAATAT | 20 | 165 | 9860-10024 |
|  |  | rev | TGGAAGTTAACGGTACTATTTATA | 24 |  |  |
| 9 | pre Arg | fwd | CTACCATGAGCCCTACAAAC | 20 | 163 | 10281-10443 |
|  |  | rev | ATGAGTCGAAATCATTCGTTT | 21 |  |  |
| 10 | pre His | fwd | AAACCCTCATTCACACGAGA | 20 | 154 | 12038-12191 |
|  |  | rev | GTAAGCCTCTGTTGTCAGATTC | 22 |  |  |
| 11 | pre Thr | fwd | GACAACCAGTAAGCTACCCTTT | 22 | 163 | 15765-15927 |
|  |  | rev | CGGTTTACAAGACTGGTGTAT | 21 |  |  |
| 12 | pre Pro | fwd | TGTACGAAATACATAGCGGTTG | 20 | 125 | 15976-16100 |
|  |  | rev | TCCACCATTAGCACCCAAAG | 22 |  |  |
| 13 | pre Glu | fwd | GGGAGGTCGATGAATGAGTG | 22 | 123 | 14689-14811 |
|  |  | rev | TACAACCACGACCAATGATATG | 20 |  |  |
| 14 | pre Ser | fwd | CGCTGCATGTGCCATTAAGA | 20 | 131 | 7470-7600 |
|  |  | rev | CCAAAGCTGGTTTCAAGCCA | 20 |  |  |
| 15 | pre Tyr | fwd | AGGTATAGTGTTCCAATGTCTTTG | 24 | 109 | 5854-5962 |
|  |  | rev | TAGATTTACAGTCCAATGCTTCAC | 24 |  |  |
| 16 | pre Gln | fwd | GGATTAATTAGTACGGGAAGGGTA | 20 | 109 | 4369-4477 |
|  |  | rev | ATTCTCCGTGCCACCTATCA | 24 |  |  |
| **Primer for adaptor and reference gene** | | | | | | |
|  | UBB | fwd | GTTGGGTGAGCTTGTTTGTG | 20 | 128 |  |
|  |  | rev | CTCAAGGGTGATGGTCTTGC | 20 |  |  |
|  | adaptor |  | CACGACGCTGTTCCATCT | 18 |  |  |
|  | heavy strand |  |  |  |  |  |
|  | light strand |  |  |  |  |  |

**Table S4** Probes for Northern blot analysis

|  |  |
| --- | --- |
| **target** | **sequence 5’ to 3’** |
| MT-12S | CAGGTCCTTTGAGTTTTAAGCTGTGGCTCGTAGTGTTCTGGCGAGCAGTTTTGTTGATTTAACTGTTGAGG |
| tRNA Ser(UCN)-rc | GAAAAAGTCATGGAGGCCATGGGGTTGGCTTGAAACCAGCTTTGGGGGGTTCGATTCCTTCCTTTTTTG |
| MT-ND4 | GCTAGTCATATTAAGTTGTTGGCTCAGGAGTTTGATAGTTCTTGGGCAGTGAGAGTGAGTAGTAGAATGT |
| MT-ND5 | ATTCCTGCTACAACTATAGTGCTTGAGTGGAGTAGGGCTGAGACTGGGGTGGGGCCTTCTATGGCTGAGG |
| MT-CO3 | ATTAGACTATGGTGAGCTCAGGTGATTGATACTCCTGATGCGAGTAATACGGATGTGTTTAGGAGTGGGAC |
| MT-CO2 | CTAGGAATAATGGGGGAAGTATGTAGGAGTTGAAGATTAGTCCGCCGTAGTCGGTGTACTCGTAGGTTCAGTACCAT |
| MT-ND1 | GTTAAAGGAGCCACTTATTAGTAATGTTGATAGTAGAATGATGGCTAGGGTGACTTCATATGAGATTGTTTG |
| tRNA Gln | TTGTAGGATGGGGTGTGATAGGTGGCACGGAGAATTTTGGATTCTCAGGATGGGTTCGATTCTCATAGTCCTAG |
| tRNA Ser(UCN) | CAAAAAAGGAAGGAATCGAACCCCCCAAAGCTGGTTTCAAGCCAACCCCATGGCCTCCATGACTTTTTC |
| 18S | GCCCCCGGCCGTCCCTCTTAATCATGGCCTCAGTTCCGAAAACCAACAAAATAGAACCGCGGTCCTATT |
| heavy strand |
| light strand |