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Data Article

Data supporting the co-expression of *PDHA1* gene and of its paralogue *PDHA2* in somatic cells of a family

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ABSTRACT

This article presents a dataset proving the simultaneous presence of a 5′UTR-truncated *PDHA1* mRNA and a full-length *PDHA2* mRNA in the somatic cells of a PDC-deficient female patient and all members of her immediate family (parents and brother).

We have designed a large set of primer pairs in order to perform detailed RT-PCR assays allowing the clear identification of both *PDHA1* and *PDHA2* mRNA species in somatic cells. In addition, two different experimental approaches were used to elucidate the copy number of *PDHA1* gene in the patient and her mother.

The interpretation and discussion of these data, along with further extensive experiments concerning the origin of this altered gene expression and its potential therapeutic consequences, can be found in "Complex genetic findings in a female patient with pyruvate dehydrogenase complex deficiency: null mutations in the *PDHX* gene associated with unusual expression

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of the testis-specific *PDHA2* gene in her somatic cells" (A. Pinheiro, M.J. Silva, C. Florindo, et al., 2016) [1].

Specifications Table

Subject area	*Biology*
More specific subject area	*Molecular Genetics*
Type of data	*Tables, figures*
How data was acquired	*Agarose gel electrophoresis after RT-PCR analyses quantitative real time PCR, microarray analyses, in silico analyses (BLAST software)*
Data format	*Raw, analyzed*
Experimental factors	*Genomic DNA and total RNA isolated from whole blood samples and fibroblast cultures*
Experimental features	*Genomic DNA was amplified by quantitative real time PCR and microarray analyses. Total RNA was reverse transcribed and amplified by semi-quantitative RT-PCR and by quantitative real time PCR using TaqMan assays. Alignment of sequences was performed using the BLAST software.*
Data source location	*Lisboa, Portugal*
Data accessibility	*Data provided within the manuscript and available in public databases (NCBI) in case of sequence alignment: GenBank accession numbers GenBank:* NM_000284.3 *(PDHA1) and GenBank:* NM_005390.4 *(PDHA2)*

Value of the data

- These data, reporting on *PDHA2* gene expression in somatic cells, may trigger new research related to the activation of a paralogue gene as a therapeutic target to loss-of-function mutations.
- Data revealing the co-existence of both *PDHA1* and *PDHA2* mRNAs in somatic cells will be useful for future experiments addressing the impact between both isoforms in the assembly of a fully functional PDC.
- Data concerning gene copy number may assist the choice of the underlying methodology.
- These dataset may contribute for designing further experiments aiming the development of alternative therapies for metabolic disorders.

1. Data

The E1 rate-limiting enzyme of pyruvate dehydrogenase complex (PDC) is a heterotetramer ($\alpha_2\beta_2$) and its α subunit is encoded by *PDHA1* gene, located in X chromosome and presenting ubiquitous expression in somatic tissues. Nevertheless a paralogue gene exists, *PDHA2*, which is located in chromosome 4 and expressed only in spermatocytes and spermatids [2].

Table 1 shows the primers used for the amplification of the analyzed genes, according to the used methodology. Fig. 1 presents the results of *PDHA1* and *PDHA2* gene expression in somatic cells of the individuals under study and in controls. Fig. 2 displays the alignment of *PDHA1* and *PDHA2* mRNAs

Table 1
List of primers used in this study.

Primer	Sequence	Position
cDNA amplification		
***PDHA1* messenger**		
PDHA1-F	5′–AGCATCCCGTAATTTTGC–3′	+75 to +92
PDHA1-R	5′–CTTTAGTTCTTCCACACTGG–3′	+989 to +1008
PDHA1-5'-F	5′–GGGCACCTGAAGGAGACTT–3′	−85 to −66
PDS1	5′–TGTGAGGAGTCGCCGCTGCC–3′	−37 to −18
PDSTr-F	5′–GCCACTGCCTGTGCTTCAT–3′	−17 to +2
PDSTr-R	5′–ACTCCATTCGGCGTACAGTCT–3′	+207 to +226
***PDHA2* messenger**		
PDHA2-F	5′–TGCCATCTACAGCACTCCGT–3′	−27 to −8
PDHA2-R	5′–CCTCCTTGAGTTGAGAACAC–3′	+1235 to +1254
***PDHX* messenger**		
PXF2	5′–CTGCTGCGTTATCTTGTGGGCT–3′	+37 to +58
PXW2	5′–TGAGTGAATGTGCCCACTGCATTG–3′	+812 to +835
PXP2	5′–CAATGCAGTGGGCACATTCACTGA–3′	+812 to +835
PXR2	5′–TAACAACTACTGAATCAACTAAGC–3′	+2060 to +2083
Genomic DNA amplification		
***PDHA1* gene**		
PDHA1-P1-F	5′–CCCTTGTTGCTTTGGTGTTT–3′	4383 to 4403
PDHA1-P1-R	5′–AGATTGCTCTGCTGACTACCG–3′	4762 to 4784
PDHA1-P2-F	5′–TGAGCATGCTGCTAATCTTCA–3′	4642 to 4682
PDHA1-P2-R	5′–CGGCGTGACAGAGTCGTAAT–3′	5114 to 5133
PDHA1-P3-F	5′–CTGGACGCCGTTCTGGTT–3′	4966 to 2983
PDHA1-P3-R	5′–GCGGAGGCGAAGTAAAGG–3′	4323 to 4340
PDHA1-P4-F	5′–TGCTTCATGAGGAAGATGCT–3′	5140 to 5159
PDHA1-P4-R	5′–AGGGTGCTGTTTGAACGAAG–3′	5526 to 5645
***PDHA2* gene**		
PDHA2-A-F	5′–GAGTAAGGAAAAGTGGAATGTCA–3′	−841 to −819
PDHA2-A-R	5′–ATCCTGCTCCATAATGTGCC–3′	−200 to −181
PDHA2-B-F	5′–GCCATCAGGATAAATGTGGC–3′	−657 to −638
PDHA2-B-R	5′–CCCTTTTCCCTGTTAAACCC–3′	−322 to −303
PDHA2-C-F	5′–AACTCTCAGAACTCTCATGTGCC–3′	−415 to −393
PDHA2-C-R	5′–ACGGAGTGCTGTAGATGGCA–3′	−27 to −8
PDHA2-D-F	5′–CAGGACCTGCCTCTATCACC–3′	−142 to +123
PDHA2-D-R	5′–AAACCGCGAATGAATTTCTG–3′	+244 to +263
PDHA2-F-F	5′–GCATGGAATTGAAGGCAGAT–3′	+212 to +231
PDHA2-F-R	5′–CCTCCTTGAGTTGAGAACAC–3′	+1298 to+1317
***PDHX* gene**		
PX1F	5′–AGAGACCTAAAGGCACCGCT–3′	+5414 to +5433
PX1R	5′–AAGCAGGCCCTCAATCATAA–3′	+5751 to +5770
PX2F	5′–TGGGAATCTTTTAGACTTTGGA–3′	+20,144 to + 20,165
PX2R	5′–TGCTGAACCCAGAAAACCTT–3′	+20,531 to + 20,550
PX3F	5′–CAACCCAGAAATAGCTACGGA–3′	+36,259 to + 36,279
PX3R	5′–CACATTAAAAATAAGGAGGCAAAA–3′	+36,557 to + 36,581
PX4F	5′–TGCAGTCATGGGGTTTTACTT–3′	+46,205 to + 46,225
PX4R	5′–ACAGCAACTTCCTACGTGATG–3′	+46,549 to +46,570
PX5F	5′–GTGACCATCTGTGGGAGTCA–3′	+49,159 to +49,173
PX5R	5′–TTATTCAGAAAACAACTCTTGCAT–3′	+49,549 to +49,573
PX6F	5′–TCACCTGCGTTTTCTGAAAGT–3′	+55,435 to + 55,456
PX6R	5′–GTGAGCCAAGATTGTGCCAT–3′	+55,779 to +55,798
PX7F	5′–TTCCACTTGTGGTTTAACGGA–3′	+58,968 to +58,988
PX7R	5′–TTTCCTCTAGCACAAATATACCCA–3′	+59,294 to +59,318
PX8F	5′–ACAAGTTTGAAGTTGTAATGGTCA–3′	+66,918 to +66,941
PX8R	5′–GAGGGAGATCAAACGATAGGA–3′	+67,178 to +67,198
PX9F	5′–TTTTTCTGTAACCGCCTTGG–3′	+73,376 to +73,395
PX9R	5′–TCTCCCCTTCACACACACAA–3′	+73,700 to +73,719

Table 1 (*continued*)

Primer	Sequence	Position
PX10F	5′–GGTAACAAAATCAAATCAAGGCA–3′	+81,064 to +81,085
PX10R	5′–TTCAGATAAATGAAAGGCTGACA–3′	+81,315 to +81,337
PX11F	5′–ACGGAAAGGGGACTTTGATT–3′	+83,725 to +83,744
PX11R	5′–TTGAGGACTAGGCAAGTCGG–3′	+84,031 to +84,050
***PDHA2* gene methylation analysis**		
CpGI-M-F	5′–ATAAATTAGTTAGTTTAGGTTGCGT–3′	−188 to −164
CpGI-M-R	5′–ATAACGTCATTTAAAAAATTACGAA–3′	+74 to +98
CpGI-U-F	5′–ATAAATTAGTTAGTTTAGGTTGTGT–3′	−188 to −64
CpGI-U-R	5′–ATAACATCATTTAAAAAATTACAAA–3′	+74 to +98
CpGII-F	5′–TGGAATTGAAGGTAGATTAGTTGTATAAAT–3′	+205 to+234
CpGII-R	5′–ATACCATTACCCCCATAAAAATTCT–3′	+406 to +431
Gene dosage analysis		
***PDHA1* gene**		
PDHA1-exon7F	5′–AGGAGGCCTTTCTGTGCTTT–3′	11,341 to 11,359
PDHA1-exon7R	5′–CGGCCCCACCACAGGGTTCCT–3′	11,616 to 11,636
***PAH* gene**		
PAH-exon1F	5′–GCTTTACTGTGCGGAGATCACCAC–3′	5315 to 5339
PAH-exon1R	5′–CTTATGAAACCAGGAAGCAC–3′	5606 to 5625

showing that the specific primers were designed to anneal to regions with null or very low homology between the two genes, thus proving the simultaneous presence of both transcripts. Fig. 3 depicts the scheme of *PDHA1* mRNA with the localization of all the primers used to prove the presence of the 5′UTR truncated *PDHA1* mRNA detected in the family samples, and to localize the truncation point. Table 2 and Fig. 4 show the results of the two different methodologies used to evaluate *PDHA1* gene copy number: quantitative real time PCR (Table 2) and microarray analyses (Fig. 4).

2. Experimental design, materials and methods

2.1. Sample preparation

Lymphocytes were isolated from three independent peripheral blood samples obtained from the index case and her parents and brother, as well as from control individuals.

Patient's fibroblast cultures were established from a diagnostic skin biopsy and grown under standard conditions.

Positive controls for *PDHA2* gene expression were obtained from two different sources; a commercially available human testis total RNA sample (Clontech Laboratories Inc., Mountain View, CA, USA) and human testis specimens from eight cases requiring open testicular biopsy for the retrieval of testicular sperm for intracytoplasmic sperm injection [3].

2.2. Nucleic acids preparation

Genomic DNA, total RNA and cDNA were prepared according to standard methods and described in [1].

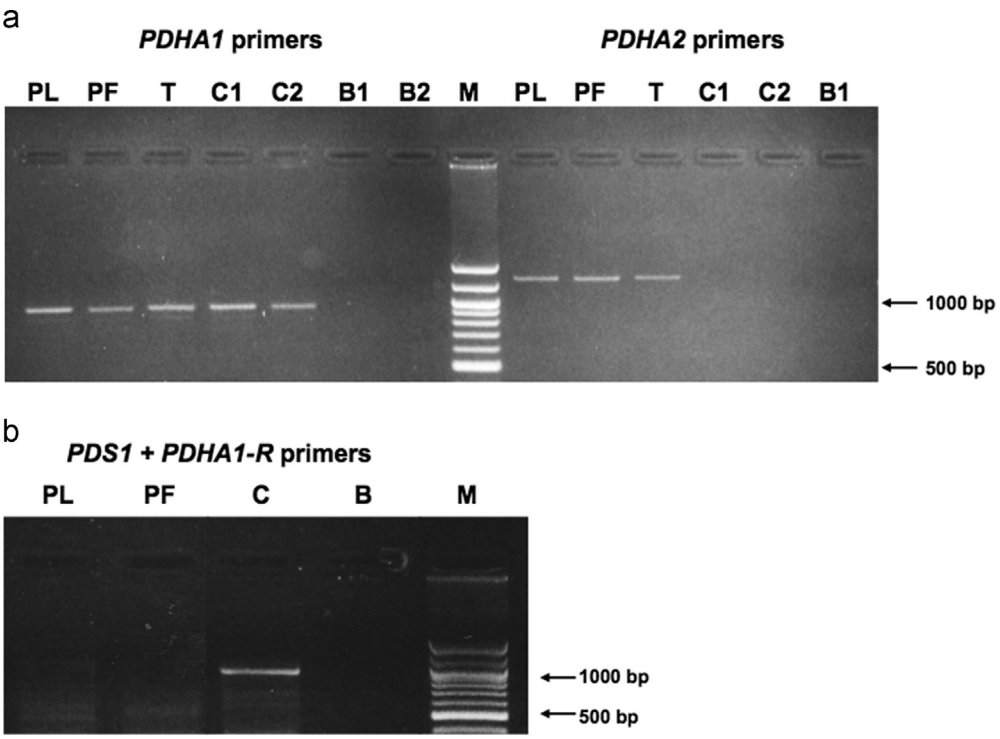

Fig. 1. RT-PCR analyses of PDH E1α transcripts. (a) Using *PDHA1* and *PDH2* specific primers. PL - patient lymphocytes; PF - patient fibroblasts; T - whole testis tissue; C1 and C2 - control lymphocytes; B1 without PCR control using whole testis total RNA; B2 - PCR control using no biological sample. M - 100 Base Pair Ladder (New England Biolabs). (b) Using forward *PDS1* primer and reverse *PDHA1* specific primer. PL - patient lymphocytes; PF - patient fibroblasts; C - control lymphocytes; B2 - PCR control using no biological sample. M - 100 Base Pair Ladder (New England Biolabs).

2.3. PCR of genomic DNA and cDNA

Amplification of the 11 individual exons of the *PDHA1* gene and related intron–exon boundaries were amplified using primers already published [4]. *PDHA1* and *PDHA2* cDNAs were amplified under conditions previously described [5] and using primers listed in Table 1, which were designed to annealing to regions displaying no homology between transcripts [6].

2.4. Evaluation of PDHA1 and PDHA2 expression and PDHA1 gene dosage

PDHA1 and *PDHA2* transcriptional levels were evaluated by quantitative real time RT-PCR under conditions previously described [1].

The copy number of *PDHA1* gene was evaluated by two methods, quantitative real time PCR and microarray analysis, as previously described [1].

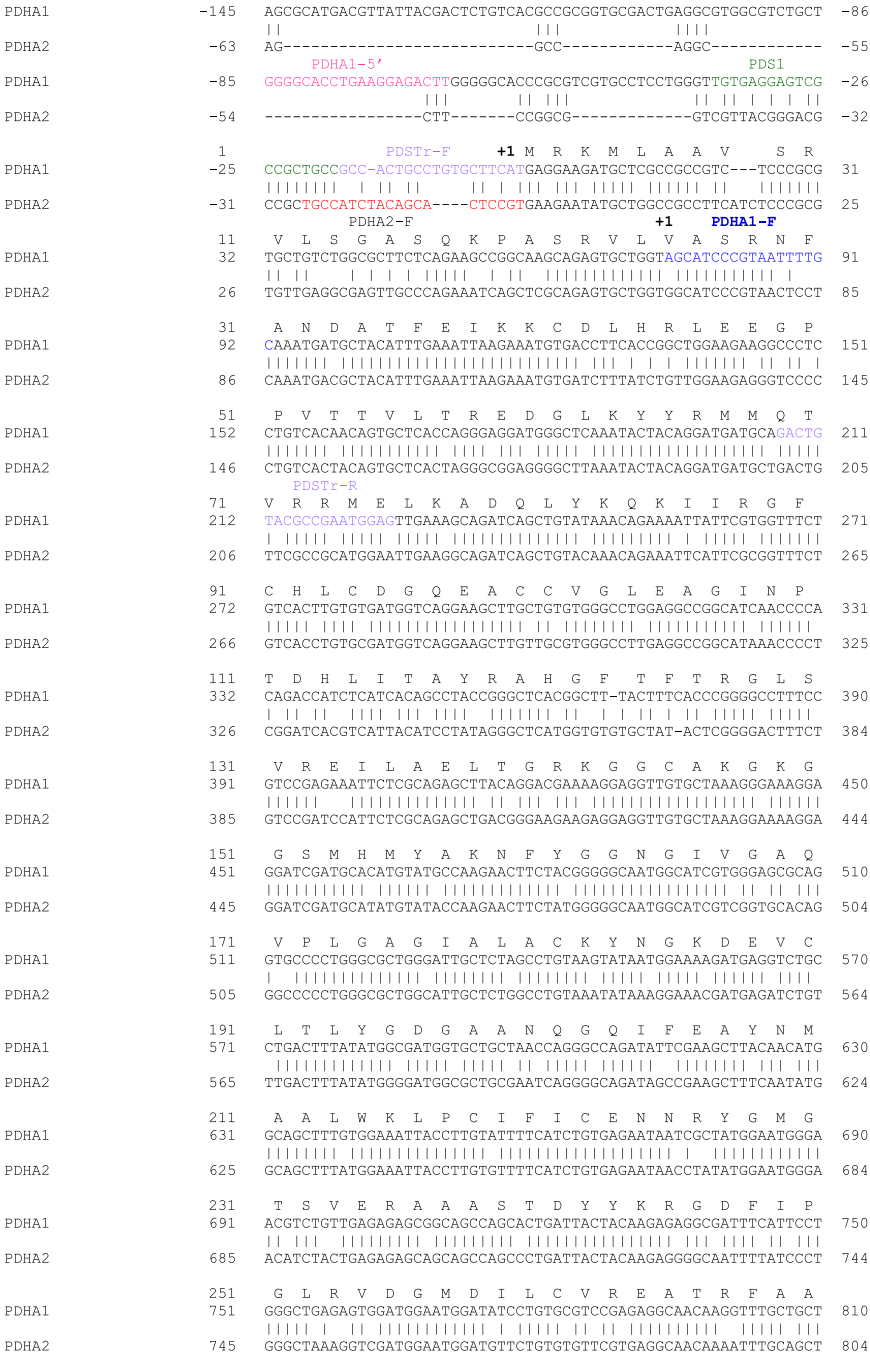

Fig. 2. Alignment of *PDHA1* and *PDHA2* cDNA sequences and primers' localization.

Fig. 2. (*continued*)

Fig. 2. (*continued*)

```
PDHA1   2971  CTGTGTAACAACAGCATAAGAATTTCTTTGTTGTAAATTTACCTTTTCAATTGTCTTTGC  3030
                                                                  ||||
PDHA2   1314  ----------------------------------------------------ATTG-------  1317

PDHA1   3031  ATCAGCTCCTTGCAGCCGCAACCAGTCTATAAGCTCTTTATCTGTTCTCTGCCCGTAGGG  3090

PDHA2         ------------------------------------------------------------

PDHA1   3091  GCCTGCTGGGTTCTCTGTAATACCTGTAACGATTGGCAATTTGTTATATATTAGTCTAAC  3150

PDHA2         ------------------------------------------------------------

PDHA1   3151  CATAAAACTCTTCAAAAGTAACCAGTTGGATTAATAAATGATTCCAGAATGTAAATGTGA  3210

PDHA2         ------------------------------------------------------------

PDHA1   3211  TGTGAAAAAGAGATGAAAAAAAAAAAAAAAAAAAA  3245
                    |||||  |
PDHA2   1318  ------AAAGACA                      1324
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Fig. 2. (*continued*)

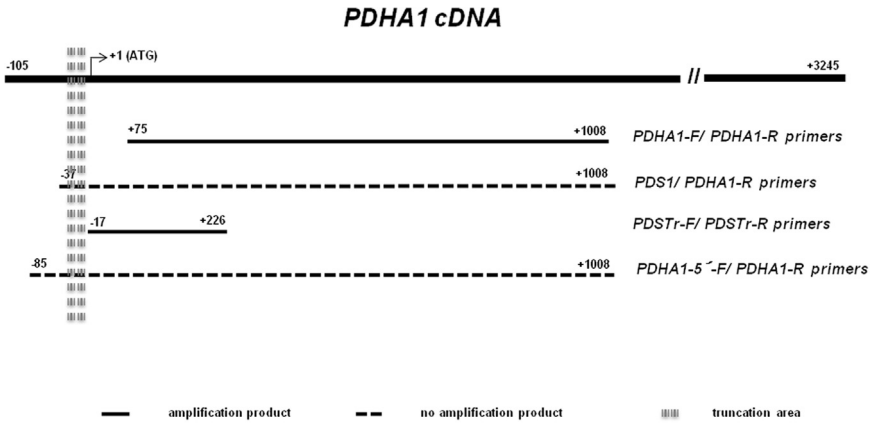

Fig. 3. Schematic representation of the *PDHA1* mRNA sequence showing the amplified *versus* non-amplified products in the RT-PCR analysis with the corresponding localization of the forward primers (PDHA1-5′, PDS1, PDSTrF, PDHA1F) and reverse primers (PDHA1R and PDSTrR), as well as the identification of the predicted truncation point.

Table 2
Calculations for determining by qPCR the copy number of *PDHA1* gene using as reference the autosomal *PAH* gene.

***PDHA1* gene**				
Sample	Ave ΔCt	ΔΔCt	RQ ($2^{-\Delta\Delta Ct}$)	Copy # ($2 \times$ RQ)
Patient	0.26	0.91	0.5	1
Control Female 1	−0.65	0	1	2
Control Female 2	−0.33	0.32	0.8	2
Control Female 3	−0.59	0.06	0.9	2
Control Male 1	0.93	1.58	0.3	1
Control Male 2	−0.23	0.42	0.7	1
Control Male 3	−0.01	0.64	0.6	1

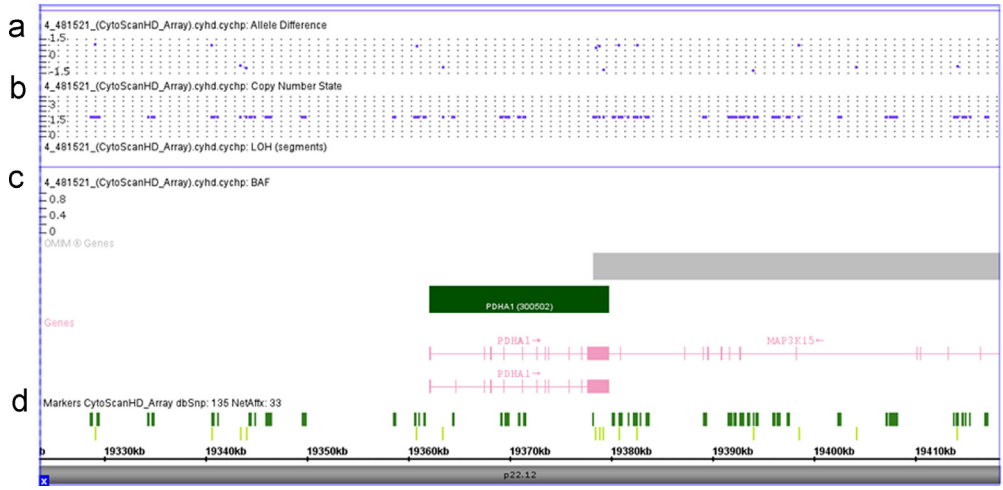

Fig. 4. Detailed view of the *PDHA1* region on chromosome X. (a) Allele difference and (b) copy number state showing absence of big deletions involving the gene. (c) OMIM genes: *PDHA1* (dark green horizontal bar) and *MAP3K15* (gray horizontal bar). Intron - horizontal pink lines; Exon - vertical pink bars. (d) Markers present in *PDHA1* region. Dark green - non-polymorphic probes; Light green - SNP, single nucleotide polymorphism. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Acknowledgments

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.08.029.

References

[1] A. Pinheiro, M.J. Silva, C. Florindo, et al., Complex genetic findings in a female patient with pyruvate dehydrogenase complex deficiency: null mutations in PDHX gene associated with unusual expression of the testis-specific PDHA2 gene in her somatic cells, Gene (2016), Jun 22 [Epub ahead of print].
[2] M.S. Patel, N.S. Nemeria, W. Furey, F. Jordan, The pyruvate dehydrogenase complexes: structure-based function and regulation, J. Biol. Chem. 289 (2014) 16615–16623.
[3] A. Pinheiro, I. Faustino, M.J. Silva, et al., Human testis-specific *PDHA2* gene: methylation status of a CpG island in the open reading frame correlates with transcriptional activity, Mol. Genet. Metab. 99 (2010) 425–430.
[4] M.J. Silva, A. Pinheiro, F. Eusébio, A. Gaspar, I. Tavares de Almeida, I. Rivera, Pyruvate dehydrogenase deficiency: identification of a novel mutation in *PDHA1* gene which responds to amino acid supplementation, Eur. J. Pediatr. 168 (2009) 17–22.
[5] W. Lissens, L. De Meirleir, S. Seneca, et al., Mutation analysis of the pyruvate dehydrogenase E1α gene in eight patients with a pyruvate dehydrogenase complex deficiency, Hum. Mutat. 7 (1996) 46–51.
[6] H.-H.M. Dahl, R.M. Brown, W.M. Hutchison, C. Maragos, G.K. Brown GK, A testis- specific form of the human pyruvate dehydrogenase E1α subunit is coded for by an intronless gene on chromosome 4, Genomics 8 (1990) 225–232.