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..."
of the testis-specific PDHA2 gene in her somatic cells" (A. Pinheiro, M.J. Silva, C. Florindo, et al., 2016) [1]. © 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Specifications Table

| Subject area       | Biology                                   |
|--------------------|-------------------------------------------|
| More specific sub- | Molecular Genetics                        |
| ject area           |                                            |
| Type of data       | Tables, figures                           |
| How data was       | Agarose gel electrophoresis after RT-PCR  |
| acquired           | analyses quantitative real time PCR,       |
|                    | microarray analyses, in silico analyses     |
|                    | (BLAST software)                          |
| Data format        | Genomic DNA and total RNA isolated from    |
|                    | whole blood samples and fibroblast cultures|
| Experimental       | Genomic DNA was amplified by quantitative  |
| factors            | 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        | Lisboa, Portugal                           |
| location           |                                            |
| 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 (PDH |
|                    | A1) 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 (α₂β₂) 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'-AGCATCCCCTATAATTTTGC-3' | +75 to +92 |
| PDHA1-R | 5'-CTTTGTTCTTCACACCTTG-3' | +989 to +1008 |
| PDHA1-5'-F | 5'-GGGCACTGAAAAGAGACT-3' | –85 to –66 |
| PDS1 | 5'-TGTGAAGAGCCCGCCTGC-3' | –37 to –18 |
| PDS1-F | 5'-GCCACTGCTTGGCTCAT-3' | –17 to +2 |
| PDS1-R | 5'-ACTCCATTGGGCTACAGTC-3' | +207 to +226 |
| **PDHA2 messenger** | | |
| PDHA2-F | 5'-TGCACTCTACAGACTGACT-3' | –27 to –8 |
| PDHA2-R | 5'-CCCTCTGTGTTGGAACAC-3' | +1235 to +1254 |
| **PDHX messenger** | | |
| PX2 | 5'-CTCTGCTGTATACGCTGTGC-3' | +37 to +58 |
| PX2W | 5'-TGAGTGAACTGACCTCCTGAT-3' | +812 to +835 |
| PX22 | 5'-CAATGGACCTCAGCAGACTATTCTA-3' | +812 to +835 |
| PX2R | 5'-TAACAACCTGTAATACGACAGC-3' | +2060 to +2083 |
| **Genomic DNA amplification** | | |
| **PDHA1 gene** | | |
| PDHA1-P1-F | 5'-CCCTTTGCTGTTTGGTGT-3' | 4383 to 4403 |
| PDHA1-P1-R | 5'-AGATGGCTCGCTGCTACTCAG-3' | 4762 to 4784 |
| PDHA1-P2-F | 5'-TGGCACTGTAATCTTCTGCA-3' | 4642 to 4682 |
| PDHA1-P2-R | 5'-CGGCTCAGAGATGCTGAAT-3' | 5114 to 5133 |
| PDHA1-P3-F | 5'-CTGAGGCGCCCTTGCTGTT-3' | 4966 to 2983 |
| PDHA1-P3-R | 5'-CGGAGGCAGATGAATTACC-3' | 4323 to 4340 |
| PDHA1-P4-F | 5'-TGCTTCTAGGGAATGCTGT-3' | 5140 to 5159 |
| PDHA1-P4-R | 5'-AGGTTGCTGTTGGAACAG-3' | 5526 to 5645 |
| **PDHA2 gene** | | |
| PDHA2-A-F | 5'-GACTAGAAGAAAATGGAGATGTA-3' | –841 to –819 |
| PDHA2-A-R | 5'-ATCTGCTCTCATAATGTCAC-3' | –200 to –181 |
| PDHA2-B-F | 5'-GCCATCAGAGATAATGGCC-3' | –657 to –638 |
| PDHA2-B-R | 5'-CCCTTTCTCCTGTGAACCC-3' | –322 to –303 |
| PDHA2-C-F | 5'-AATCTCTAAGACCATCTTGG-3' | –415 to –393 |
| PDHA2-C-R | 5'-ACGGATTGCTAGTTAGCC-3' | –27 to –8 |
| PDHA2-D-F | 5'-CAGAGCTCTGTGCTACCTAC-3' | –142 to +123 |
| PDHA2-D-R | 5'-AAACCAGGAGATGAATCCT-3' | +244 to +263 |
| PDHA2-F-F | 5'-CATGGAATGAGAGCCGAT-3' | +212 to +231 |
| PDHA2-F-R | 5'-CCCTCTTCAGTGCTAAGAC-3' | +1298 to +1317 |
| **PDHX gene** | | |
| PX1F | 5'-AGACACCTAAGGGCCAGCG-3' | +5414 to +5433 |
| PX1R | 5'-AAGACGCCCTCAATACATA-3' | +5751 to +5770 |
| PX2F | 5'-TGGAATCTTTTATGGACTCTG-3' | +20,144 to +20,165 |
| PX2R | 5'-TGGCAAGCGCAATAACCC-3' | +20,531 to +20,550 |
| PX3F | 5'-CAACCTAGAGATAAATTGGA-3' | +36,259 to +36,279 |
| PX3R | 5'-CATTTAAAATAAAGGAGCGAAA-3' | +36,557 to +36,581 |
| PX4F | 5'-TGACGCTCGGCGTTTTTAC-3' | +46,205 to +46,225 |
| PX4R | 5'-ACAGAACTCCCTGCAGTG-3' | +46,549 to +46,570 |
| PX5F | 5'-GTGACACCTTCTGTGGCAGT-3' | +49,159 to +49,173 |
| PX5R | 5'-TTATTCGAGAAAACATCCTGC-3' | +49,549 to +49,573 |
| PX6F | 5'-TCACCTGCTGTGTTCTCAGAC-3' | +54,435 to +55,456 |
| PX6R | 5'-TGACGCCAGATTGGTCTCC-3' | +55,779 to +55,798 |
| PX7F | 5'-TCTCCATTGCTGTGTTCTCAG-3' | +58,968 to +58,988 |
| PX7R | 5'-TTGCCTGAGAAATATTACCC-3' | +59,294 to +59,318 |
| PX8F | 5'-ACAAGTTTTGTAATGGTCTCA-3' | +66,918 to +66,941 |
| PX8R | 5'-GAGGAGTCAAGAATCTGGA-3' | +67,178 to +67,198 |
| PX9F | 5'-TTTTCGTTACCCGCTTGG-3' | +73,376 to +73,395 |
| PX9R | 5'-TCTCCATCAGACACACAA-3' | +73,700 to +73,719 |
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].
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)
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Fig. 2. (continued)
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 PDStR), 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.

| Sample      | Ave ΔCt | ΔΔCt | RQ (2^ΔΔCt) | Copy # (2 × 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 | 1.1         | 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).

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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.

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