# Pyruvate dehydrogenase complex deficiency: updating the clinical, metabolic and mutational landscapes

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**Research**

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## Abstract

Background : Pyruvate dehydrogenase complex (PDC) catalyzes the irreversible decarboxylation of pyruvate into acetyl-CoA which ultimately generates ATP. PDC deficiency can be caused by alterations in any of the genes encoding its several subunits, and the resulting phenotype, though very heterogeneous, mainly affects the neuro-encephalic system. The aim of this study is to describe and discuss the clinic, metabolic and genotypic profiles of thirteen PDC deficient patients, thus seeking to establish possible genotype-phenotype correlations.

Results : The mutational spectrum revealed that seven patients (54 %) carry mutations in the PDHA1 gene , encoding the E1α subunit, five patients (38 %) carry mutations in the PDHX gene, encoding the E3 binding protein, and the remaining patient (8 %) harbors mutations in the DLD gene, encoding the E3 subunit. These data corroborate PDHA1 mutations as the predominant cause of PDC deficiency, though revealing a notable prevalence of PDHX mutations among Portuguese patients, most of them carrying a seemingly private mutation (p.R284X). The biochemical analyses revealed high lactate and pyruvate plasma levels whereas de ratio L/P was under 16; enzymatic activities, when compared to control values, revealed to be independent from the genotype and ranged from 8.5% to 30% which may be considered a cut-off value for primary PDC deficiency. Concerning the clinical features, all patients displayed developmental delay/psychomotor retardation, the severity of which seems to correlate with the type and localization of the mutation carried by the patient. The therapeutic options essentially go through the administration of a ketogenic diet and supplementation with thiamine, although arginine aspartate intake revealed to be beneficial in some patients. Moreover, the in silico analysis of the missense mutations present in this PDC deficient population allowed to understand the molecular mechanism underlying these pathogenic variants.

Conclusion : The identification of the disease-causing mutations, together with the functional and structural characterization of the mutant protein variants, allows to get insight on the severity of the clinical phenotype and the selection of the most appropriate therapy.


## Background

Pyruvate dehydrogenase complex (PDC) deficiency, first described in 1970 by Blass and colleagues (1), is an inborn error of mitochondrial energy metabolism. The pyruvate oxidation route, that bridges the cytosolic glycolytic pathway and the mitochondrial tricarboxylic acid cycle (2), involves not only PDC but also pyruvate transport and the ancillary metabolic routes associated with various cofactors. PDC, which irreversibly decarboxylates pyruvate to acetyl coenzyme A, comprises three functional (E1 or pyruvate dehydrogenase, E2 or dihydrolipoamide transacetylase, and E3 or dihydrolipoyl dehydrogenase) and one structural (E3BP or E3 binding protein, formerly designated by protein X) components, and the regulatory PDC kinases and PDC phosphatases, which control the complex activity via phosphorylation/dephosphorylation (3). The E1 subunit catalyzes the first, rate-limiting and irreversible step of the reaction which takes place in two active sites formed at the interface between their α and β subunits, each requiring a thiamine pyrophosphate cofactor and magnesium ions for activity (4,5).

The great majority (77.3%) of patients with PDC deficiency harbor mutations in the X-linked *PDHA1* gene which encodes the E1α subunit. Hemizygous males are usually symptomatic whereas heterozygous females present variable expression due to random patterns of X-inactivation in different tissues. Therefore, the clinical manifestations depend on the proportion of cells expressing the mutant E1α subunit. The remaining cases are caused by mutations in the *PDHB* gene encoding the E1β subunit (4.3%), in the *DLAT* gene encoding the E2 subunit (1.5%), in the *DLD* gene encoding the E3 subunit (6.2%) and finally in the *PDHX* gene encoding the E3BP subunit (10.7%) (6).

The clinical presentation is extremely heterogeneous, ranging from a fatal lactic acidosis and progressive neurological and neuromuscular degradation in the neonatal period to a chronic neurological dysfunction and neurodegenerative condition. Inadequate removal of lactate but mainly of pyruvate (7) results in lactic acidemia which, together with the blood lactate/pyruvate ratio ≤20, is recognized as an important biomarker (8,9). The primary phenotypic manifestation corresponds to an impairment of neurological and/or developmental functions, leading to a wide range of symptoms and clinical features: hypotonia, seizures, structural and functional brain abnormalities, ataxia, respiratory distress (like apnea and hypoventilation), facial dysmorphism, and peripheral neuropathy. According to their severity, the phenotypes can be divided into three main categories: neonatal, infantile and benign (10–12).

The existing therapeutic strategies target: i) the metabolic pathway, bypassing energy production via a ketogenic diet (11), ii) the regulatory system of the dysfunctional PDC, using xenobiotic inhibitors (13,14), or iii) cofactor(s) stimulation of the residual PDC activity with supplementation of these (15,16). Nevertheless, none of these treatments is sufficiently effective and the responsiveness is markedly individual. Thus, the precise diagnosis, including the identification of the genetic defect and the phenotypic consequences, is becoming increasingly decisive for selecting the appropriate therapeutic strategy. Knowledge on the functional and structural impact of the identified mutations on the resulting PDC component variant provides relevant information for informed decisions on the therapeutic approaches.

In this study, we describe and discuss the profiles of thirteen Portuguese PDC deficient patients with reference to their clinical data, biochemical findings and results of DNA analysis, thus seeking to establish possible genotype-phenotype correlations. This is the first report on phenotypic and genotypic variability in a subset of PDC deficient patients diagnosed in Portugal.

## Results

*General information*

The broad phenotypic spectrum of PDC deficiency, common to many other genetic mitochondrial disorders, often hampers the achievement of a rapid diagnosis. Although among the patients suspected to be PDC deficient based on clinical signs /symptoms and biochemical date, namely elevated plasma

lactate (L) and pyruvate (P) levels with low L/P ratio and / or impaired PDC activity, we only considered those whose genetic diagnosis was accomplished. Accordingly, a total of 13 individuals, all of European ancestry, were enrolled in this study (Table 1).

The 13 patients, seven males and six females, were born between 1983 and 2018, their current ages varying from 20 months to 36 years, with a median age of 15.5 years. Consanguinity was reported only in three families harboring *PDHX* mutations. Among this group of 13 patients, only two were siblings, born from a triplet pregnancy after *in vitro* fertilization (two affected males and one unaffected female) who carry a *PDHA1* mutation. No affected relatives are known in any of these families.

All patients showed the first symptoms either in the neonatal period (five individuals) or during infancy (eight individuals). However, in some cases, the diagnosis was only achieved later, mainly among the older patients who presented a less severe clinical picture; the most striking example is the deficiency in E3 (also designated by dihydrolipoamide dehydrogenase, DLD), which was only diagnosed when the patient was 17 years old.

All patients but one are alive; the deceased patient is a boy carrying the c.1132C>T mutation in *PDHA1* (generating the p.R378C E1α variant); the death occurring at 3.3 years and being caused by cardiorespiratory arrest.

## Genetic findings

This cohort of 13 patients revealed a mutational spectrum encompassing ten different mutations distributed by three different genes: five in *PDHA1,* three in *PDHX* and two in *DLD.* The results revealed that seven patients carried mutations in *PDHA1* (~54 %), five carried mutations in *PDHX* (~38%), and one carried mutations in *DLD* (~8 %) (Table 1).

It is interesting to note that most patients with mutations in *PDHA1,* presenting an X-linked inheritance pattern, are males (6/7) in whose families no consanguinity was reported, mutations likely arrived in all by a *de novo* mechanism, and mosaicism was absent. On the contrary, most patients with mutations in *PDHX,* displaying an autosomal recessive mode of inheritance, are females (4/5) and consanguinity was detected in three cases. Finally, the single patient with mutations in *DLD,* also displaying an autosomal recessive mode of inheritance, is a compound heterozygous female with no consanguinity reported in her family.

Concerning the type of mutations found in these patients, their severity is also predictably different according to the affected gene. Indeed, a single mutant allele in *PDHA1* is sufficient to cause PDC deficiency, including in females who carry two alleles. In this patient cohort, all five different *PDHA1* mutations are missense (c.615C>G, c.757A>G, c.905G>A, c.1132C>T, and c.1133G>A), generating respectively the p.F205L, p.R253G, p.R302H, p.R378C and p.R378H variants. The prevalence of missense mutations, in principle the less severe mutation type, strongly correlates with the majority of patients being males. On the contrary, the three mutations detected in *PDHX* gene (c.160+1G>A, c.483delC and c.850C>T), being nonsense (p.R284X) and frameshift (p.G26Vfs*7 and p.P161Pfs*17), usually result in absent protein, hence their highly deleterious consequences. Three out of the five patients are homozygous for the p.R284X variant, whereas one of the remaining patients is a compound heterozygote expressing the p.R284X and p.P161Pfs*17 variants, and the other is homozygous for the mutation generating the p.G26Vfs*7 variant. Regarding the two identified *DLD* mutations, both c.259C>T and c.803_804delAG are novel (Exome Variant, LOVD or ClinVar). The c.259C>T mutation generates the E3 p.P87S variant. Moreover, c.803_804delAG, originating a frameshift variant (p.Q268Rfs*3), is predicted to be very severe.

## Biochemical findings

All patients, regardless of the carried mutation, were suspected of PDC deficiency by their elevated plasma lactate and pyruvate levels, ranging from 3.0 to 17.0 mmol·L$^{-1}$ and from 0.27 to 0.81 mmol·L$^{-1}$, respectively. Lactate/pyruvate ratios, as expected, were always ≤16, i.e. in the normal range with a single exception. PDC deficiency was confirmed by determination of PDC activity. Enzymatic assays were performed either with circulating lymphocytes (12 patients) or cultured fibroblasts (two patients), both tissues having been analyzed in patient 11. All patients had their enzymatic activities confirmed in a second independent sample and the results always matched. Interestingly, a single false negative result was firstly determined and precisely in lymphocytes of a female patient later identified with a *PDHA1* mutation. In our experience, the cut-off value for considering a primary PDC deficiency should be ≤ 30% of control activity and, indeed, all patients but one presented enzymatic activities ranging from 8.5% to 30%. The single exception was one of the siblings carrying the p.R378C variant in *PDHA1,* who presented 40 % of normal control activity (Table 1), surprisingly the one whose symptoms manifested earliest in the neonatal period.

## Clinical features

The clinical features observed in these patients' cohort are displayed in Table 1 and, according to the most recently published data (6), can be roughly divided into two categories: one caused by *PDHA1* and *PDHX* mutations, and the other caused by *DLD* mutations. Indeed, the clinical phenotypes manifested by patients carrying *PDHA1* and *PDHX* mutations are quite variable and almost undistinguishable.

In the latter group, concerning neurological features, all individuals presented development delay/psychomotor retardation, which ranged from severe (eight patients) to moderate (three patients) or very mild (one patient who attended normal school). Hypotonia was also observed in all individuals, with a single exception (Patient 11) who nevertheless could display it at an earlier age. Seizures were reported for half the individuals, especially those carrying *PDHA1* mutations (5/7 patients). On the contrary, ocular manifestations were more frequent in individuals harboring *PDHX* mutations (4/5 patients). Microcephaly, dystonia and ataxia were also reported in half the patients of both mutational groups. As expected, facial dysmorphism was only detected in a single female

patient carrying a *PDHA1* mutation. The patient with DLD deficiency revealed moderate developmental delay/psychomotor retardation, seizures, hypotonia, dystonia and ataxia. Basal ganglia abnormalities were exclusively observed in patients carrying mutations in the *PDHA1* gene. Cerebral atrophy, but not cerebellar atrophy, was detected mainly in patients carrying *PDHX* mutations. However, among patients carrying *PDHA1* mutations, cerebral atrophy, when present, was always associated with cerebellar atrophy (Figures 1 and 2). The individual harboring mutations in the *DLD* gene (Patient 13) presented partial agenesis of *corpus callosum.*

# Treatment

All the patients are under therapeutic measures which encompass the most frequent modalities: ketogenic diet, thiamine supplementation and antiepileptic drugs. Three patients with *PDHA1* mutations and three with *PDHX* mutations are under ketogenic diet with perceived clinical benefits. The long-term thiamine supplementation, as PDC cofactor, is prescribed in almost all patients (11/13), and antiepileptics drugs only in those presenting seizures (Table 1). Finally, it is interesting to mention that three of the patients carrying mutations in the *PDHA1* gene were under arginine aspartate supplementation, with beneficial effects.

# *In silico* analysis of missense mutations

Bioinformatic analysis using the PolyPhen–2 server (17) suggested that all mutations but one affecting E1α subunit are most probably damaging. The E1α p.R302H, p.R378C and p.R378H variants displayed a score of 1.000 (sensitivity 0.00 and specificity 1.00), p.F205L displayed a score of 0.919 (sensitivity 0.81 and specificity 0.94), and p.R253G displayed a score of 0.007 (sensitivity 0.96 and specificity 0.75) thus being considered benign. As for E3 subunit, the p.P87S variant is predicted to be most probably damaging, with a score of 0.996 (sensitivity 0.55 and specificity 0.98).

To complement the information of the PolyPhen–2 server, which solely bases its predictions on the polypeptide sequence and overlooks other structural and functional details, such as e.g. cofactor binding and interaction with other proteins, we obtained and thoroughly inspected structural models of each E1α variant. The models obtained for the E1α p.R253G, p.R378C, p.R378H and p.F205L variants have been recently described by our group, attempting to understand the molecular mechanisms underlying the pathogenicity of the corresponding mutations (18). All mutations result in putative loss of H-bonds, electrostatic or hydrophobic interactions between the side chains of the substituted amino acids and neighboring residues, with predicted effects on P-loop destabilization, inter-subunit interactions and proper oligomeric assembly, as well as interaction with other PDC components. Herein, we additionally generated a structural model of the E1α p.R302H variant (Figure 3). R302 is located at one end of the P-loop A, its side chain being within electrostatic and H-bonding distance to the side chain or main chain carbonyl moieties of Y287, R288, Y289, H290 (active site residue) and G298, all residues belonging to the same P-loop. Upon substitution by a histidine residue, most of the possible interactions between its side chain and other residues in the P-loop are lost. The single remaining H bond is that between the side chain imidazole and the main chain carbonyl of G298. Therefore, in the p.R302H E1α variant, the net loss of four possible side chain interactions with other P-loop A residues (Figure 3) is likely to contribute to a more disordered loop and consequently lower enzymatic activity. Notably, the degree of disorder in P-loop A has been negatively correlated with E1 enzymatic activity, since an ordered loop favors TPP binding, which itself promotes P-loop A order (19).

To further understand the pathogenicity of the *DLD* mutation generating the E3 p.P87S variant, a structural model was obtained (Figure 4) based on the reported 3D crystallographic structure of another disease-causing E3 variant (PDB entry 6I4T) (20). As observed in Figure 4, P87 is located in a helix that lines with the flavin adenine dinucleotide isoalloxazine ring and contains the active site disulfide composed of C45 and C50. Substitution of P87 by a serine is likely to alter the flexibility and thus the overall stability of the respective helix, possibly affecting the enzymatic activity.

## Discussion

PDC deficiency diagnosis is extremely challenging due to a phenotypic presentation that can be observed in many other disorders, especially those causing altered mitochondrial metabolism (6,21,22). Given this context, we aimed to present the first report on Portuguese PDC deficient patients combining information on the associated clinical, biochemical, enzymatic and genotypic spectra. The mutational spectrum of PDC deficiency in this group of patients revealed ten different mutations affecting three genes, *PDHA1* (54 %), *PDHX* (38 %) and *DLD* (8 %). Concerning each gene deficiency prevalence, these data generally agree with literature surveys (6,9) and also with several studies focused on different populations (8,23,24). However, the frequency of mutations in the *PDHA1* gene is lower than the usually reported average of 75–80 %.

The most striking evidence is the relatively high incidence of E3BP deficiency, mostly caused by a single *PDHX* variant, p.R284X, half the cases originating from the Azores Islands. This E3BP variant was first described by our group (25) and, until now, only another Portuguese patient has been reported to carry this mutation (26). Furthermore, the mutational spectrum of E3BP deficiency in Portugal includes very severe mutations, leading to null alleles. Nevertheless, the older patients surprisingly reached adulthood, in line with the high proportion of long-term survival among reported E3BP deficient patients (8,27,28). In general, an overwhelming majority of the mutations hitherto identified in the *PDHX* gene are, as in this work, deletions, nonsense mutations, point mutations at intron-exon boundaries, or even large intra-genic rearrangements, expected to result in a complete absence of E3BP protein (8,28–30). Despite this fact, the patients retain considerably significant PDC activity (20–30 %), taking into account the expected impairment on PDC assembly. On the one hand, as a structural subunit devoid of enzymatic activity, E3BP does not directly contribute to the complex catalytic activity. On the other hand, the significantly truncated E3BP, if present at all in the cell, would likely compromise the structure of the E2/E3BP PDC core and binding of the E3 component. Both E2 and E3BP components have a similar structure and domain organization, despite only E2 being catalytically active (31). However, the possibility of the E2 core directly binding to the E3 enzyme may underlie the observed residual PDC activity (28–30,32). In their recent work, Prajapati and collaborators report a non-uniform stoichiometry of the E2/E3BP PDC core. The imbalanced distribution of E2 and E3BP constituents of the trimeric units results into structurally

dynamic E1 and E3 clusters (33). Moreover, for one of the proposed models of *E. coli,* PDC core is a fully functional E2 homotrimer operating in a “division-of-labor” mechanism, including binding of E3 component (33,34).

Concerning the mutational spectrum of E1α deficiency, five different *PDHA1* mutations were identified but a single one (p.R378C) in non-consanguineous patients. Almost all mutations affect an arginine codon (35) and those situated in exons 10 and 11 cause a severe phenotype, because the resulting protein variants present very low enzyme activity. On the contrary, the two mutations located in exon 7 originate moderate (p.F205L) or very mild (p.R253G) phenotypes. Interestingly, mutations affecting codon 378 are considered particularly lethal (9). Indeed, from our male patients carrying the p.R378C mutation, one deceased at three years of age and the twins, presently aged 8 years, display a severe clinical picture. However, a female patient bearing the p.R378H substitution reached the adulthood, probably due to a lyonization effect.

Regarding a possible genotype-dependent phenotypic presentation, our data is roughly suggestive of such a correlation. Effectively, the patients harboring the most deleterious mutations (p.R302H and p.R378C in *PDHA1* gene, and all the mutations in *PDHX* gene) present the most severe phenotypes, involving serious psychomotor retardation, hypotonia and seizures, whereas those carrying less severe mutations accordingly display a better clinical outcome. The most puzzling observation concerns the female carrying mutations in *DLD* gene. Despite being a compound heterozygote bearing two severe mutations, her clinical course was satisfying until 2018 when she suffered an acute metabolic decompensation originating spastic tetraparesis with gait and language loss. Although she partially recovered language, currently she presents only a moderate to severe psychomotor handicap. Despite the E3 subunit being common to other enzyme complexes, such as α-ketoglutarate dehydrogenase and branched-chain amino acid dehydrogenase, this patient did not display the associated biochemical or clinical phenotypes.

Irrespectively of our patients cohort size, the majority of our PDC deficient patients remarkably reached adulthood, as opposed to several other reports (9,23,24,36). Concerning the therapeutic measures to which these individuals are subjected, it is clear they are only palliative, since all but one patient continue presenting clinical features ranging from moderate to severe forms. The single exception is Patient 2 who seems to represent an exceptional case, because his treatment only encompasses thiamine (E1 subunit cofactor) and arginine aspartate (potential protein stabilizer) supplementation (37). Indeed, this patient carried the p.R253G mutation that originates an E1α subunit whose *in silico* and *in vitro* analyses with the recombinant E1 variant exhibited proneness to aggregation and low enzymatic activity.

In conclusion, the identification of the disease-causing mutations, together with the functional and structural characterization of the mutant protein variants, allows to get insight on the severity of the clinical phenotype and the selection of the most appropriate therapy, namely the option for a ketogenic diet.

## Materials And Methods

*Cohort of patients*

This study included all PDC deficient patients whose diagnosis was confirmed at the molecular level: thirteen individuals comprising a pair of monozygous twin siblings, 46% being females and 54% males. Since patients originated from all regions of Portugal, this cohort is representative of the whole population. Diagnosis of patients, suspected by high lactate and pyruvate plasma levels and respective lactate/pyruvate ratio < 20, was confirmed by reduced PDC activity (*ca* <30 % of laboratory control mean: 1734 ± 455 pmol·min$^{-1}$·mg protein$^{-1}$; range: 1279—2189; n = 70) in peripheral lymphocytes and/or cultured fibroblasts originating from skin biopsy, and also by identification of the causative mutation(s).

This study was approved by the local Ethics Committees and informed consents were obtained from the patients or their parents, who were also enrolled in the study, whenever necessary and possible. Declaration of Helsinki was also strictly observed.

*Phenotypic evaluation*

The physicians following these patients completed a questionnaire involving a wide range of parameters, namely: general characteristics of the patients, clinical features, brain malformations, biochemical findings, genetic findings, and current therapy.

# Sample Collection and Preparation

Blood samples were collected after overnight fasting by venipuncture into EDTA-containing tubes for plasma separation and quantification of lactate and pyruvate levels, and into heparin-containing tubes for peripheral blood mononuclear cells (PBMC) isolation.

PBMC were separated at room temperature on a Ficoll-Paque gradient. Fibroblasts were cultured in Dulbecco’s Modified Eagle Medium supplemented with 10% newborn calf serum and 1% antibiotic/antimycotic solution. Pelleted cells were resuspended in homogenization buffer (80 mM $KH_2PO_4$, pH 7.4, 2 mM EDTA). PBMC suspensions were immediately disrupted by sonication, whereas fibroblast suspensions were treated with 5 mM dichloroacetate for 15 minutes at 37 °C. The reaction was blocked by addition of a stopping solution (25 mM NaF, 25 mM EDTA, 4 mM DTT), and cells were disrupted by three freeze/thaw cycles.

# PDC Activity Assay

Enzymatic activity was measured using a radiochemical method based on the release of $^{14}CO_2$ from [1–$^{14}$C]-pyruvate (38) with minor modifications (Johannes Mayr and Wolfgang Sperl, personal communication). Briefly, 100 µL of cell homogenates were incubated at 37 °C for 10 minutes in 100 µL of

reaction buffer (32 mM phosphate buffer containing 4 mM $MgCl_2$, 2 mM $CaCl_2$, 0.5 mM $NAD^+$, 0.5 mM thiamine pyrophosphate (TPP), 0.1 mM CoA and 5 mM carnitine; final concentrations); blanks were obtained by replacing cell homogenate with homogenizing buffer. Then, the reaction was started by addition of 50 µL [1–$^{14}$C]-pyruvate solution (0.5 mM, 0.067 µCi) and allowed to proceed for 30 minutes, after which the reaction was stopped by addition of 80 µL 6N $H_2SO_4$. The released $^{14}CO_2$ was trapped in filter paper saturated with benzethonium hydroxide, for 15 minutes post-incubation at room temperature and under gentle stirring, and its amount measured in a scintillation counter. All samples were analyzed in triplicates and PDC activity was expressed in pmol·min$^{-1}$·mg protein$^{-1}$.

## Preparation of Genomic DNA, RNA and cDNA

Genomic DNA and, eventually, total RNA were isolated from peripheral blood leukocytes using the Puregene Cell and Tissue kit (Gentra Systems) and the Trizol method, respectively; 5 g of total RNA were used for the reverse transcription reaction (Amersham First Strand cDNA Synthesis kit, GE Healthcare Bio-Science Corp.).

## PCR amplification of PDC coding genes

The complete sequence of each gene was obtained by PCR amplification of individual exons, including intronic boundaries, or overlapping fragments of the respective cDNA. The PDC subunits under analysis together with their coding genes, approved symbols and reference sequences are listed in Table S1, whereas primer sequences designed for each gene amplification are listed in Table S2.

## Sequence Analysis

PCR and RT-PCR products were purified from solution or directly from agarose gels, using Isolate II PCR and Gel Kit (Bioline). PCR forward or reverse primers were added to the purified products from each individual sample and submitted to bi-directional Sanger sequencing. All chromatograms corresponding to PCR and RT-PCR fragments were analyzed using the BLAST program (NCBI).

## *In silico* analysis of PDC-E1 mutations

To better establish genotype-phenotype correlations regarding the missense mutations in *PDHA1,* we undertook an *in silico* analysis of protein variants resulting from the described mutations. Besides evaluating the potential pathogenicity of the mutations using the PolyPhen–2 server (17), we sought to obtain structural models of the protein variants through two complementary strategies, previously described for the p.R253G, p.R378C, p.R378H, and p.F205L variants (18), and herein extended to p.R302H: i) submitting the sequence of the amino acid substituted variant to the SwissModel server and retrieving the corresponding model; and ii) using the Mutagenesis tool in Pymol (version 1.7) (39) employing the structure of WT PDC-E1 (PDB entry 3EXE) as template (19).

## Declarations


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### Data Availability

Data can be made available upon reasonable request to the corresponding authors.

### AUTHORS' CONTRIBUTION

Design of the study: IR, JBV, ITA, HP-P. Experimental analyses: HP-P, MJS, CF, IR. Cliical data collection and follow-up of patients: SS, ACF, SD, ALR, PJ, AO, DG, AB, EM, RG SS. Writing and reviewing of the manuscript: IR, JBV, ITA, HP-P. All authors read and approved the final version of the manuscript.

### ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All procedures were in accordance with the ethical standards of responsible committee on human experimentation, institutional and national, and with Principles of the Declaration of Helsinki. Informed consent was obtained from all patients or their parents/guardians.

### CONSENT FOR PUBLICATION

Consent was obtained from all patients for publication.

### COMPETING InterestS

The Authors declare they have no conflict of interest.

## References


1. Blass JP, Avigan J, Uhlendorf BW. A defect in pyruvate decarboxylase in a child with an intermittent movement disorder. J Clin Invest [Internet]. 1970 Mar 1 [cited 2014 May 27];49(3):423–32. Available from: http://www.jci.org/articles/view/106251
2. Patel MS, Harris RA. Mammalian alpha-keto acid dehydrogenase complexes: gene regulation and genetic defects. FASEB J. 1995 Sep;9(12):1164–72.
3. Patel MS, Korotchkina LG. Regulation of the pyruvate dehydrogenase complex. Biochemical Society Transactions [Internet]. 2006 Apr 1 [cited 2019 Aug 27];34(2):217–22. Available from: http://www.biochemsoctrans.org/bst/034/bst0340217.htm
4. Cate RL, Roche TE, Davis LC. Rapid intersite transfer of acetyl groups and movement of pyruvate dehydrogenase component in the kidney pyruvate dehydrogenase complex. J Biol Chem [Internet]. 1980 Aug 25 [cited 2019 Sep 15];255(16):7556–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6772637
5. Berg A, Westphal AH, Bosma HJ, De Kok A. Kinetics and specificity of reductive acylation of wild-type and mutated lipoyl domains of 2-oxo-acid dehydrogenase complexes from Azotobacter vinelandii. Eur J Biochem [Internet]. 1998 Feb 15 [cited 2019 Sep 15];252(1):45–50. Available from: http://doi.wiley.com/10.1046/j.1432-1327.1998.2520045.x
6. Sperl W, Fleuren L, Freisinger P, Haack TB, Ribes A, Feichtinger RG, et al. The spectrum of pyruvate oxidation defects in the diagnosis of mitochondrial disorders. J Inherit Metab Dis [Internet]. 2015 May 20 [cited 2019 Apr 8];38(3):391–403. Available from: http://doi.wiley.com/10.1007/s10545-014-9787-3
7. Debray F-G, Mitchell GA, Allard P, Robinson BH, Hanley JA, Lambert M. Diagnostic Accuracy of Blood Lactate-to-Pyruvate Molar Ratio in the Differential Diagnosis of Congenital Lactic Acidosis. Clin Chem [Internet]. 2007 May 1 [cited 2020 May 11];53(5):916–21. Available from: https://academic.oup.com/clinchem/article/53/5/916/5627488
8. Imbard A, Boutron A, Vequaud C, Zater M, de Lonlay P, de Baulny HO, et al. Molecular characterization of 82 patients with pyruvate dehydrogenase complex deficiency. Structural implications of novel amino acid substitutions in E1 protein. Mol Genet Metab [Internet]. 2011 Dec [cited 2014 May 16];104(4):507–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21914562
9. Patel KP, O'Brien TW, Subramony SH, Shuster J, Stacpoole PW. The spectrum of pyruvate dehydrogenase complex deficiency: clinical, biochemical and genetic features in 371 patients. Mol Genet Metab [Internet]. 2012 Jul [cited 2014 May 25];106(3):385–94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4003492&tool=pmcentrez&rendertype=abstract
10. Brown GK, Otero LJ, LeGris M, Brown RM. Pyruvate dehydrogenase deficiency. J Med Genet [Internet]. 1994 Nov 1 [cited 2019 Aug 27];31(11):875–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7853374
11. Wexler ID, Hemalatha SG, McConnell J, Buist NR, Dahl HH, Berry SA, et al. Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations. Neurology [Internet]. 1997 Dec [cited 2014 May 27];49(6):1655–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9409363
12. Robinson JN, Norwitz ER, Mulkern R, Brown SA, Rybicki F, Tempany CMC. Prenatal diagnosis of pyruvate dehydrogenase deficiency using magnetic resonance imaging. Prenat Diagn [Internet]. 2001 Dec [cited 2019 Nov 10];21(12):1053–6. Available from: http://doi.wiley.com/10.1002/pd.187
13. Fouque F, Brivet M, Boutron A, Vequaud C, Marsac C, Zabot M-T, et al. Differential effect of DCA treatment on the pyruvate dehydrogenase complex in patients with severe PDHC deficiency. Pediatr Res [Internet]. 2003 May [cited 2014 May 9];53(5):793–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12621116
14. Ferriero R, Brunetti-Pierri N. Phenylbutyrate increases activity of pyruvate dehydrogenase complex. Oncotarget. 2013;4(6):804–5.
15. Pastoris O, Savasta S, Foppa P, Catapano M, Dossena M, Ornella P. Pyruvate dehydrogenase deficiency in a child responsive to thiamine treatment. Acta Paediatr Int J Paediatr. 1996;85(5):625–8.
16. Naito E, Ito M, Yokota I, Saijo T, Matsuda J, Ogawa Y, et al. Thiamine-responsive pyruvate dehydrogenase deficiency in two patients caused by a point mutation (F205L and L216F) within the thiamine pyrophosphate binding region. Biochim Biophys Acta - Mol Basis Dis. 2002 Oct 9;1588(1):79–84.
17. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Vol. 7, Nature Methods. 2010. p. 248–9.
18. Pavlu-Pereira H, Silva MJ, Tomé CS, Florindo C, Tavares de Almeida I, Leandro P, et al. Molecular determinants of pathogenicity in clinically relevant E1α variants causing pyruvate dehydrogenase complex deficiency. Submitt manuscript, under Rev. 2020;
19. Whitley MJ, Arjunan P, Nemeria NS, Korotchkina LG, Park Y-H, Patel MS, et al. Pyruvate dehydrogenase complex deficiency is linked to regulatory loop disorder in the αV138M variant of human pyruvate dehydrogenase. J Biol Chem [Internet]. 2018 Aug 24 [cited 2019 Apr 8];293(34):13204–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29970614
20. Szabo E, Wilk P, Nagy B, Zambo Z, Bui D, Weichsel A, et al. Underlying molecular alterations in human dihydrolipoamide dehydrogenase deficiency revealed by structural analyses of disease-causing enzyme variants. Hum Mol Genet. 2019 Jul 23;
21. Ciara E, Rokicki D, Halat P, Karkucińska-Więckowska A, Piekutowska-Abramczuk D, Mayr J, et al. Difficulties in recognition of pyruvate dehydrogenase complex deficiency on the basis of clinical and biochemical features. The role of next-generation sequencing. Mol Genet Metab reports [Internet]. 2016 Jun [cited 2019 Aug 27];7:70–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214426916300167
22. Shin HK, Grahame G, McCandless SE, Kerr DS, Bedoyan JK. Enzymatic testing sensitivity, variability and practical diagnostic algorithm for pyruvate dehydrogenase complex (PDC) deficiency. Mol Genet Metab [Internet]. 2017 Nov [cited 2019 Apr 8];122(3):61–6. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/28918066

23. Barnerias C, Saudubray JM, Touati G, De Lonlay P, Dulac O, Ponsot G, et al. Pyruvate dehydrogenase complex deficiency: Four neurological phenotypes with differing pathogenesis. Dev Med Child Neurol [Internet]. 2010 Feb [cited 2019 Nov 21];52(2):e1–9. Available from: http://doi.wiley.com/10.1111/j.1469-8749.2009.03541.x
24. Quintana E, Gort L, Busquets C, Navarro-Sastre A, Lissens W, Moliner S, et al. Mutational study in the PDHA1 gene of 40 patients suspected of pyruvate dehydrogenase complex deficiency. Clin Genet [Internet]. 2010 May [cited 2019 Aug 27];77(5):474–82. Available from: http://doi.wiley.com/10.1111/j.1399-0004.2009.01313.x
25. Pinheiro A, Silva MJ, Pavlu-Pereira H, Florindo C, Barroso M, Marques B, et al. 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. Gene. 2016;591(2):417–24.
26. Nogueira C, Silva L, Pereira C, Vieira L, Leão Teles E, Rodrigues E, et al. Targeted next generation sequencing identifies novel pathogenic variants and provides molecular diagnoses in a cohort of pediatric and adult patients with unexplained mitochondrial dysfunction. Mitochondrion. 2019 Jul 1;47:309–17.
27. Marsac C, Stansbie D, Bonne G, Cousin J, Jehenson P, Benelli C, et al. Defect in the lipoyl-bearing protein X subunit of the pyruvate dehydrogenase complex in two patients with encephalomyelopathy. J Pediatr [Internet]. 1993 Dec 1 [cited 2019 Oct 16];123(6):915–20. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0022347605803877?via%3Dihub
28. Brown RM, Head RA, M AA, J JA, Walter JH, Whitehouse WP, et al. Pyruvate dehydrogenase E3 binding protein (protein X) deficiency. Dev Med Child Neurol [Internet]. 2007 Feb 13 [cited 2019 Nov 26];48(9):756–60. Available from: http://doi.wiley.com/10.1111/j.1469-8749.2006.tb01362.x
29. Byron O, Lindsay JG. The pyruvate dehydrogenase complex and related assemblies in health and disease. In: Sub-Cellular Biochemistry [Internet]. 2017 [cited 2019 Aug 27]. p. 523–50. Available from: http://link.springer.com/10.1007/978-3-319-46503-6_19
30. Gray LR, Tompkins SC, Taylor EB. Regulation of pyruvate metabolism and human disease. Cell Mol Life Sci [Internet]. 2014 Jul 21 [cited 2019 Aug 27];71(14):2577–604. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24363178
31. Harris RA, Bowker-Kinley MM, Wu P, Jeng J, Popov KM. Dihydrolipoamide dehydrogenase-binding protein of the human pyruvate dehydrogenase complex. DNA-derived amino acid sequence, expression, and reconstitution of the pyruvate dehydrogenase complex. J Biol Chem. 1997 Aug 8;272(32):19746–51.
32. Vijayakrishnan S, Callow P, Nutley MA, McGow DP, Gilbert D, Kropholler P, et al. Variation in the organization and subunit composition of the mammalian pyruvate dehydrogenase complex E2/E3BP core assembly. Biochem J [Internet]. 2011 Aug 1 [cited 2014 May 26];437(3):565–74. Available from: http://www.biochemj.org/bj/437/0565/bj4370565.htm
33. Prajapati S, Haselbach D, Wittig S, Patel MS, Chari A, Schmidt C, et al. Structural and Functional Analyses of the Human PDH Complex Suggest a “Division-of-Labor” Mechanism by Local E1 and E3 Clusters. Structure. 2019 Jul 2;27(7):1124-1136.e4.
34. Song J, Jordan F. Interchain acetyl transfer in the E2 component of bacterial pyruvate dehydrogenase suggests a model with different roles for each chain in a trimer of the homooligomeric component. Biochemistry [Internet]. 2012 Apr 3 [cited 2020 Jan 21];51(13):2795–803. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22413895
35. Vitkup D, Sander C, Church GM. The amino-acid mutational spectrum of human genetic disease. Genome Biol [Internet]. 2003 Oct 30 [cited 2020 Feb 19];4(11):R72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14611658
36. DeBrosse SD, Okajima K, Zhang S, Nakouzi G, Schmotzer CL, Lusk-Kopp M, et al. Spectrum of neurological and survival outcomes in pyruvate dehydrogenase complex (PDC) deficiency: Lack of correlation with genotype. Mol Genet Metab. 2012 Nov;107(3):394–402.
37. Silva MJ, Pinheiro A, Eusébio F, Gaspar A, Tavares de Almeida I, Rivera I. Pyruvate dehydrogenase deficiency: identification of a novel mutation in the PDHA1 gene which responds to amino acid supplementation. Eur J Pediatr [Internet]. 2009 Jan [cited 2014 May 28];168(1):17–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18398624
38. Clot JP, Benelli C, Fouque F, Bernard R, Durand D, Postel-Vinay MC. Pyruvate dehydrogenase activity is stimulated by growth hormone (GH) in human mononuclear cells: a new tool to measure GH responsiveness in man. J Clin Endocrinol Metab [Internet]. 1992 Jun [cited 2019 Nov 10];74(6):1258–62. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.74.6.1592868
39. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 2018 Jul 2;46(W1):W296–303.

## Table

**Table 1. Genetic, biochemical, clinical and therapeutics data concerning the cohort of 13 Portuguese PDC deficient patients.**

| Patient | 1-JCF | 2-MJG | 3-DMN | 4-GCN* | 5-RCN* | 6-JAR | 7-ARV | 8-GMR | 9-MJP | 10-LCM | 11-AVB | 12-MBS | 13-MMS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **General information** | | | | | | | | | | | | | |
| Year of birth | 1996 | 2000 | 2005 | 2011 | 2011 | 2015 | 1983 | 2002 | 2018 | 2015 | 1988 | 2004 | 2001 |
| Gender | M | M | M | M | M | M | F | M | F | F | F | F | F |
| Consanguinity | N | N | NA | N | N | N | N | Y | Y | N | N | Y | N |
| Age of onset (years) | 1.9 | 2 | Neonatal period | 0.6 | Neonatal period | 0.5 | 1 | 0.5 | Neonatal period | 0.5 | Neonatal period | Neonatal period | 0.4 |
| Age of diagnosis (years) | 9 | 5.3 | 0.4 | 0.6 | 0.6 | 1 | 6 | 2 | Neonatal period | 3.3 | Neonatal period | 0.5 | 17 |
| Actual age (years) | 23 | 19 | 14 | 8 | 8 | Deceased at 3.3 | 36 | 17 | 1 | 4 | 31 | 15 | 18 |
| **Genetic findings** | | | | | | | | | | | | | |
| Affected gene | *PDHA1* | *PDHA1* | *PDHA1* | *PDHA1* | *PDHA1* | *PDHA1* | *PDHA1* | *PDHX* | *PDHX* | *PDHX* | *PDHX* | *PDHX* | *DLD* |
| Affected protein component | E1α | E1α | E1α | E1α | E1α | E1α | E1α | E3BP | E3BP | E3BP | E3BP | E3BP | E3 |
| Nucleotide exchange | c.615C>G | c.757A>G | c.905G>A | c.1132C>T | c.1132C>T | c.1132C>T | c.1133G>A | c.850C>T / c.850C>T | c.850C>T / c.850C>T | c.850C>T / c.850C>T | c.850C>T / c.483delC | c.160+1G>A / c.160+1G>A | c.259C>T / c.803_804delAG |
| Precursor protein Exchange | p.F205L | p.R253G | p.R302H | p.R378C | p.R378C | p.R378C | p.R378H | p.R284X / p.R284X | p.R284X / p.R284X | p.R284X / p.R284X | p.R284X / p.P161Pfs*17 | p.G26Vfs*7 / p.G26Vfs*7 | p.P87S / p.Q268Rfs*3 |
| Localization of mutation | Exon 7 | Exon 7 | Exon 10 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 7 | Exon 7 | Exon 7 | Exon 7/Exon 4 | Intron 1 | Exon 4/Exon 9 |
| Mutation type | Missense | Missense | Missense | Missense | Missense | Missense | Missense | Nonsense | Nonsense | Nonsense | Nonsense / Frameshift | Splicing | Missense / Frameshift |
| **Biochemical Findings** | | | | | | | | | | | | | |
| Highest plasma lactate (mmol·L$^{-1}$) | 7.9 | 4.0 | 9.1 | 9.4 | 6.6 | 8.9 | 4.5 | 3.5 | 17.0 | 3.0 | 4.4 | 12.2 | 3.0 |
| Highest plasma pyruvate (mmol·L$^{-1}$) | 0.527 | 0.300 | 0.398 | NA | 0.810 | 0.631 | 0.274 | 0.331 | 0.610 | 0.270 | 0.270 | 0.569 | NA |
| Ratio L/P in same sample | 14 | 12 | 20 | NA | 8 | 14 | 16 | 16 | 14 | 9 | 16 | 16 | NA |
| Enzyme activity in lymphocytes | 30 | 13 | 25** | 28 | 40 | 8.5 | 19 | 30 | 25 | 30 | 21.3 / 16.3** | 29 | 29 |
| **Clinical Features** | | | | | | | | | | | | | |
| DD/MR/PMR | Moderate | Mild | Severe | Severe | Severe | Moderate | Moderate | Severe | Severe | Severe | Severe | Severe | Moderate |
| Hypotonia | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | N | Y | Y |
| Seizures | N | N | Y | Y | Y | Y | Y | Y | N | N | N | N | Y |
| Microcephaly | Y | N | Y | N | N | N | Y | N | Y | Y | N | N | N |
| Dystonia | Y | N | Y | Y | Y | Y | N | Y | N | N | Y | Y | Y |
| Ataxia | Y | Y | N | Y | Y | N | N | Y | N | N | Y | Y | Y |
| Peripheral neuropathy | Y | Y | NA | N | N | N | N | N | N | N | N | N | N |
| Facial dysmorphisms | N | N | N | N | N | N | Y | N | N | N | N | N | N |
| Spasticity | Y | N | Y | Y | Y | N | N | N | N | N | N | N | N |
| Respiratory distress | N | N | Y | N | Y | Y | N | N | N | N | N | N | N |
| Ocular manifestations | N | N | Cortical blindness | N | N | N | N | Strabismus | Nystagmus, loss of visual acuity | Loss of visual acuity | N | Nystagmus, strabism | Astigmatism |
| **Brain Malformations** | | | | | | | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Basal ganglia abnormalities | Y | N | Y | Y | Y | Y | N | N | N | N | N | N | N |
| Cerebral brain atrophy | Y | N | Y | N | N | N | N | Y | Y | Y | N | N | Y |
| Cerebellar brain atrophy | Y | N | Y | N | N | N | N | N | N | N | N | N | N |
| **Therapy** | | | | | | | | | | | | | |
| Ketogenic diet | N | N | N | Y | Y | Y | N | Y | Y | Y | N | Y | N |
| Thiamine | N | Y | N | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Arginine aspartate | N | Y | N | N | N | N | N | N | Y | Y | N | N | N |
| Antiepiletic drugs | N | N | Y | Y | Y | Y | Y | N | N | N | N | N | Y |

## Figures

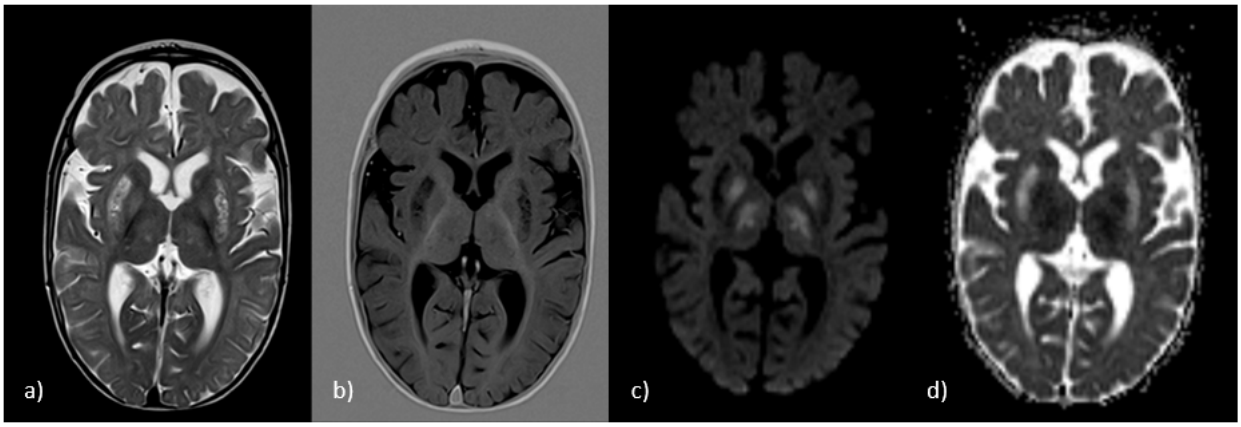


**Figure 1**

Magnetic resonance images of brain lesions of an 8 month old patient carrying a PDHA1 mutation. Hyperintense bilateral and symmetrical lesions in the thalami, globus pallidus and putamina, on T2 weighted images, hypointense on T1 and with diffusion restriction suggestive of acute lesions; there is no diffusion restriction suggestive of chronic lesions. a) axial T2 b) axial T1 IR c) axial DWI d) axial ADC.

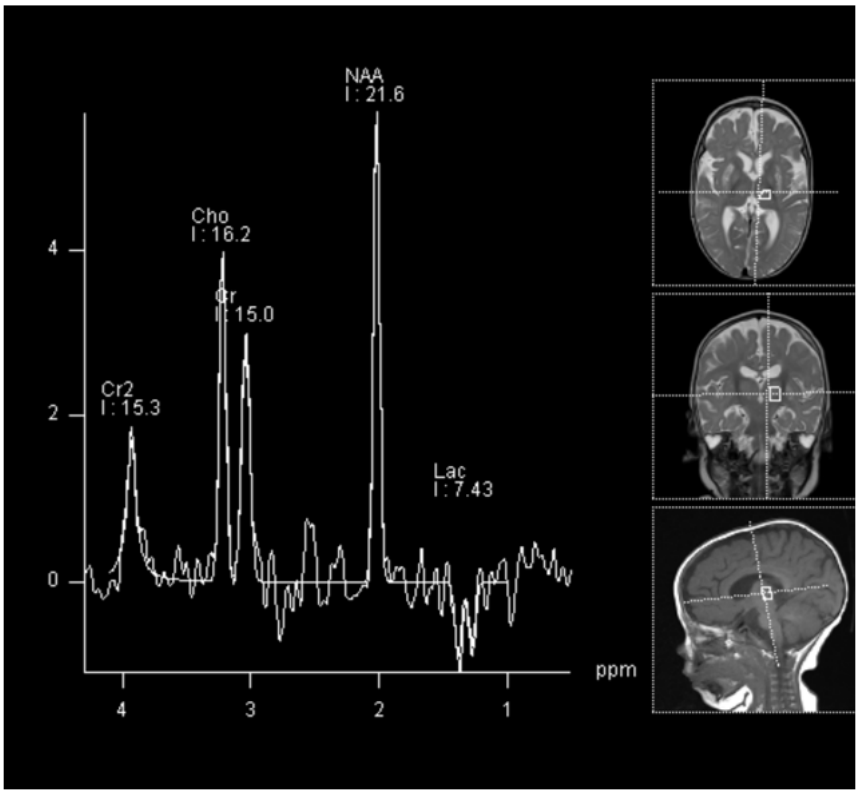


**Figure 2**

Magnetic resonance spectroscopy of a patient carrying a PDHA1 mutation. Spectroscopy with TE = 135 ms in left thalamus; slight reduction of NAA, increased choline and lactate.

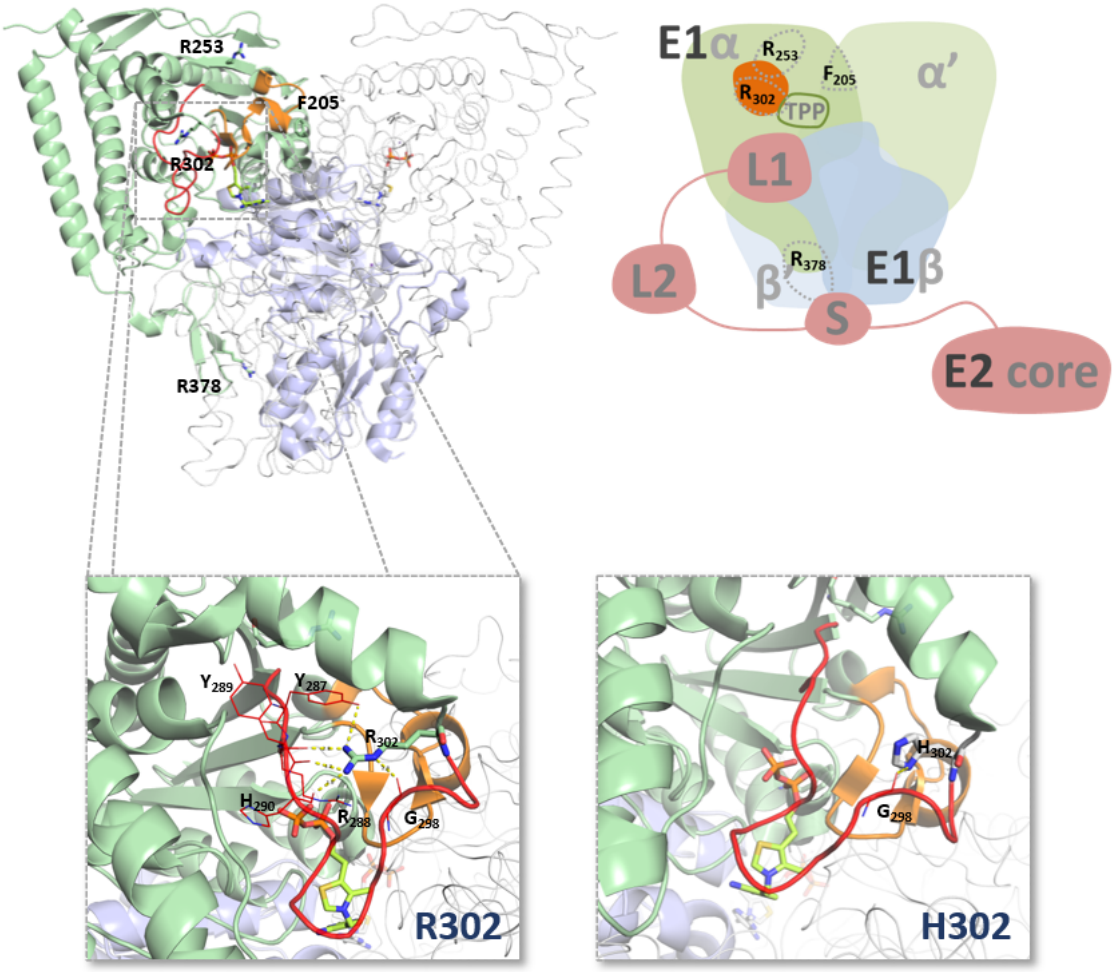


**Figure 3**

In silico analysis of pyruvate dehydrogenase complex E1 p.R302H variant. Top left panel, cartoon and ribbon representation of the heterotetrameric PDC E1 crystallographic structure (PDB entry 3EXE). E1α subunit represented in green; E1β represented in blue; E1α phosphorylation loop A represented in orange. The corresponding E1α' and E1β' subunits are represented as grey ribbon. Residues that are substituted in variants identified in Portuguese PDC deficient patients with mutations in PDHA1, encoding the PDH E1α subunit, are represented in sticks. Top right panel, scheme representing the possible impact of substituted residues in pathogenic E1α variants: p.R253G substitution located near phosphorylation loop A (orange shape); p.F205L substitution possibly affecting αα' interface (each E1α subunit represented in different shades of green); p.R302H substitution located in phosphorylation loop A close to the TPP cofactor binding site; p.R378C/H substitutions located close to the αβ interface (each E1β subunit represented in different shades of blue), and possibly affecting the interaction with a domain of the PDC E2 component. Bottom panel, zoom-in on the region surrounding R302 (left), showing its possible interactions with neighbouring residues (Y287, R288, Y289, H290 and G298), only the latter being retained upon substitution by H in the p.R302H variant (right). Structural model of PDC-E1 p.R302H variant was obtained by loading the structure of WT PDC-E1 (PDB entry 3EXE) into Pymol and applying the mutagenesis tool to generate all possible rotamers of the substituting amino acid side chain.

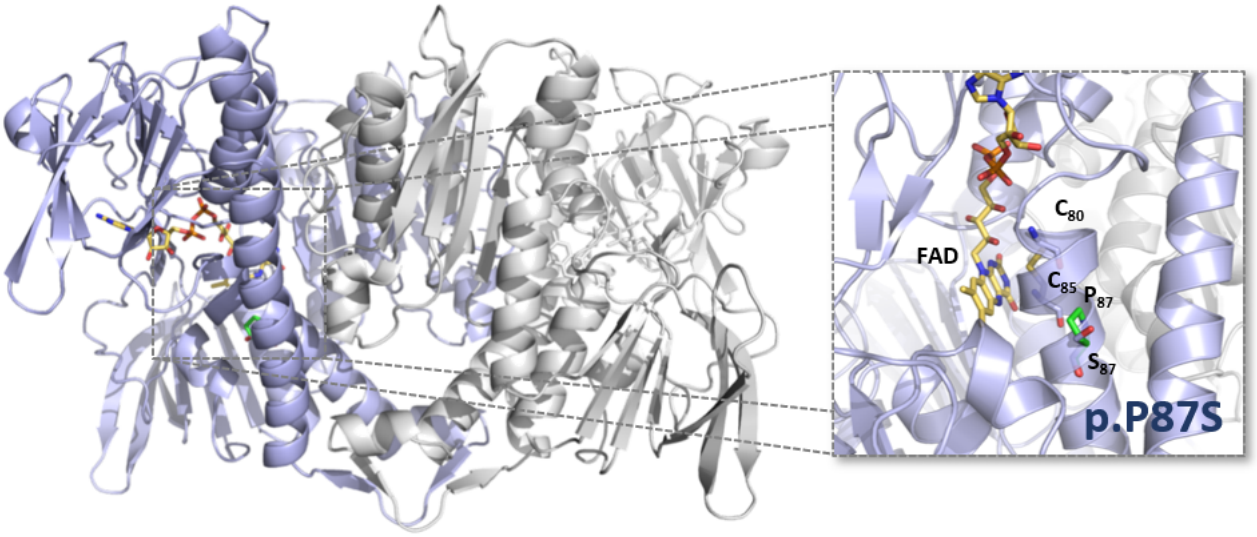


**Figure 4**

In silico analysis of pyruvate dehydrogenase complex E3 p.P87S variant. Cartoon representation of homodimeric PDC E3 crystallographic structure (PDB entry 6I4T; one monomer represented in blue, the other in grey). Flavin adenine dinucleotide (FAD) cofactor in yellow sticks. Right panel, zoom-in on the location of the P87S substitution. P87 is located in an α-helix which contains the active site cysteine residues C80 and C85. Substitution of P by S will likely affect the helix structure and disturb the proximity between the active site disulphide and the FAD cofactor. Structural model of PDC-E1 p.R302H variant was obtained by loading the structure of WT PDC-E3 (PDB entry 6IT4) into Pymol and applying the mutagenesis tool to generate all possible rotamers of the substituting amino acid side chain.

## Supplementary Files

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- SupplementaryMaterial.docx