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Case Report

Somatic mosaicism for a novel *PDHA1* mutation in a male with severe pyruvate dehydrogenase complex deficiency

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ABSTRACT

Pyruvate dehydrogenase complex (PDC) deficiencies are mostly due to mutations in the X-linked *PDHA1* gene. Males with hemizygous *PDHA1* mutations are clinically more severely affected, while those with mosaic *PDHA1* mutations may manifest milder phenotypes. We report a patient harboring a novel, mosaic missense *PDHA1* mutation, c.523G > A (p.A175T), with a severe clinical presentation of congenital microcephaly, significant brain abnormalities, persistent seizures, profound developmental delay, and failure to thrive. We review published cases of *PDHA1* mosaicism.

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1. Introduction

The mitochondrial multi-enzyme pyruvate dehydrogenase complex (PDC) is the gateway for oxidative metabolism of carbohydrates, catalyzing oxidative decarboxylation of pyruvate into acetyl-CoA as the primary substrate for the tricarboxylic acid cycle and oxidative phosphorylation. It is comprised of multiple catalytic components: including E1α (encoded by *PDHA1*), E1β, E2, and E3 subunit proteins; and the vitamin coenzyme thiamine pyrophosphate (TPP) [1]. PDC deficiency is a

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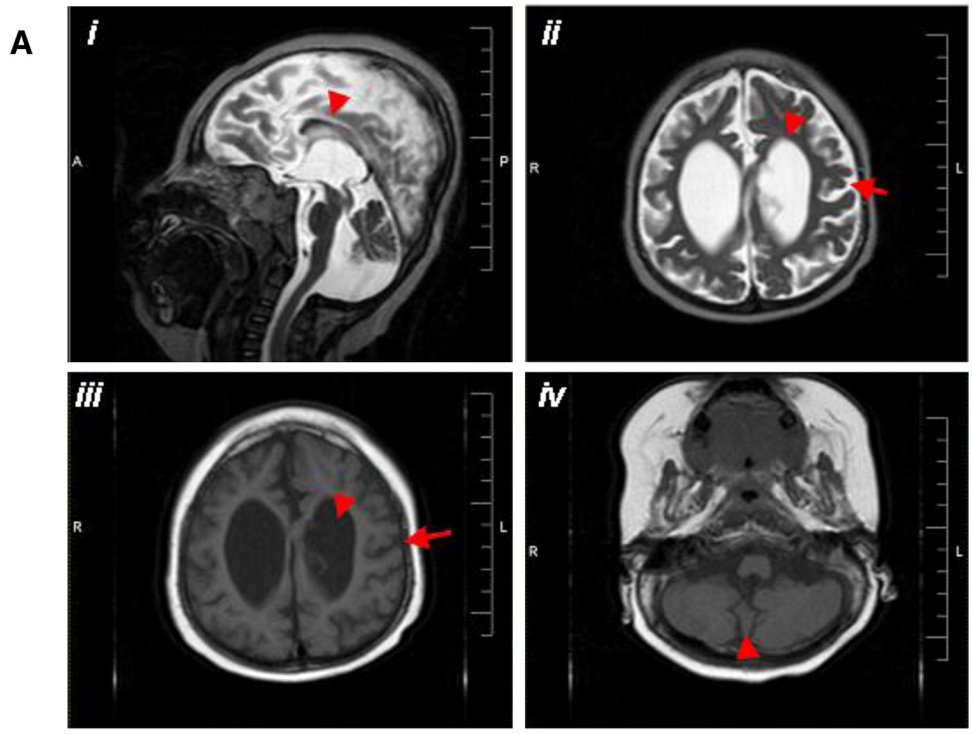
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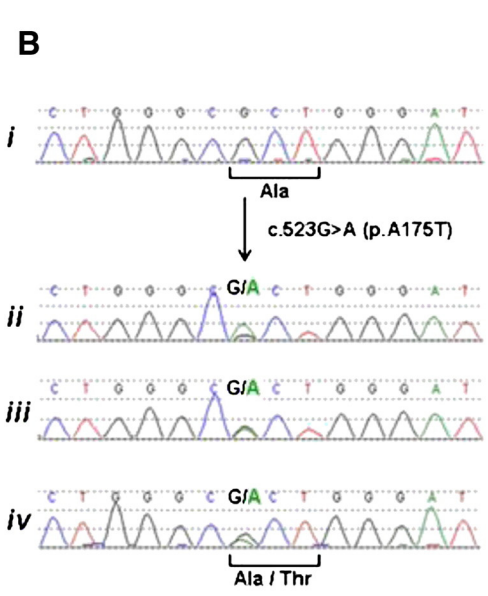
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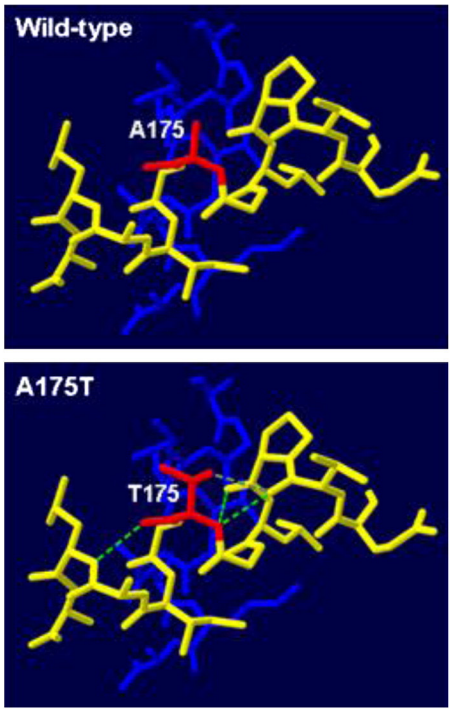
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major cause of primary lactic acidemia. The clinical presentation of PDC deficiencies are quite variable and may include severe neonatal lactic acidosis (often with early lethality), neurological involvement ranging from intermittent ataxia to persistent seizures, developmental delay, structural brain anomalies, and degenerative encephalopathy [1–3].

About 90% of PDC deficiencies in genetically confirmed patients result from mutations in the X-linked *PDHA1* [2,3]. More than 100 different mutations have been described [2–7]. Although similar numbers of affected males and females have been identified, there is a disproportionate distribution of mutation types between genders [2,3,6]. Deletion/insertion mutations in exons 10 and 11, which result in premature termination codons, are often observed in females, whereas missense/nonsense mutations in exons 3, 7, 8 and 11 are predominant in males [6]. Hemizygous males with deleterious *PDHA1* mutations are often clinically more severely affected (including lethality in infancy). Females with the same mutation may show variable clinical manifestations and greater survival due to skewed X-inactivation [1–3]. Mosaicism of *PDHA1* mutations has been documented in only a few patients.

We report a case of *PDHA1* mosaicism in a male patient with functional PDC deficiency. This is the seventh reported case of male mosaicism for *PDHA1* mutation; and this patient notably has a severe clinical phenotype.

2. Case report

This male child was born to a healthy non-consanguineous couple with normal pregnancy and delivery. At birth, the infant was microcephalic, hypotonic, and required some ventilatory support. Brain CT scan MRI revealed marked hydrocephalus with partial agenesis of the corpus callosum and colpocephaly. At 14 months of age, an intercurrent respiratory illness precipitated eye deviations and tonic–clonic movements of his upper extremities. Seizures were confirmed by EEG and he was started on antiepileptic medications. Repeat brain MRI at two years of age revealed severe hypoplasia of the corpus callosum, ventriculomegaly, hypoplasia of the cerebellar vermis consistent with the Dandy Walker variant, marked volume loss of the brain parenchyma and prominence of the cortical sulci, and absence of the cavum septum pellucidum (Fig. 1A). Follow-up evaluations revealed severe microcephaly with bitemporal narrowing and a shallow, sloping forehead, and no progression of developmental milestones. Karyotype and chromosomal microarray analysis were normal. Testing for storage disorders, peroxisomal disorders, purine processing disorders, and disorders of creatine processing and transport were all negative. Family history was non-contributory.

3. Results

The patient exhibited persistent lactic acidosis and hyperalaninemia with normal lactate to pyruvate ratio, suggestive of PDC deficiency. Biochemical analysis revealed metabolic acidosis (bicarbonate 10 mmol/L; reference range (RR) 17–29), elevated blood lactate (3.5–5.5 mmol/L, n = 10; RR 0.5–2.2), pyruvate (0.34–0.35 mmol/L, n = 2; RR 0.03–0.08), and alanine (565 μmol/L; RR: 143–439) levels with normal lactate to pyruvate ratio (ranging 10–13). Increased lactate and pyruvate were also noted in urine specimens. Activity of PDC in cultured skin fibroblasts (SFs) was 26% and 31% of the mean (0.63 and 0.76 nmol/min/mg protein; control mean 2.42; range 1.26–4.42 (3rd–97th %tile); n = 329).

Fig. 1. MRI showing brain anomalies, sequence analysis and protein structure prediction of *PDHA1* with c.523G > A (p.A175T) mutation in our patient. A. Brain MRI with and without contrast of our patient at 27 months of age: T2 sagittal image showing severely hypoplastic corpus callosum (arrowhead) (A-*i*); T2 and T1 axial images, respectively, showing marked ventriculomegaly (arrowhead) and marked loss of volume of the brain parenchyma with marked prominence of the cortical sulci (arrow) (A-*ii and iii*); and T1 axial image showing a component of cerebellar vermian hypoplasia (arrowhead), consistent with Dandy-Walker variant (A-*iv*). No abnormalities noted in the basal ganglia and there was absence of the cavum septum pellucidum (not shown here). **B.** Sequence chromatograms of *PDHA1* showing a normal sequence in the proband's mother's blood (B-*i*); mosaicism for c.523G > A (p.A175T) in the proband's cultured SFs (B-*ii*); the proband's blood (B-*iii*); and the proband's buccal cells (B-*iv*). Note the difference in mosaic ratio among samples; the peak heights vary in forward and reverse sequences and are not quantitative. **C.** *In silico* prediction of altered protein structure of *PDHA1* c.523G > A (p.A175T) mutation (red) based on Protein Data Bank entry 3EXE (Swiss-pdbViewer 4.1.0, http://spdbv.vital-it.ch). Side-chains and polypeptide backbone of E1α and E1β are depicted in yellow and blue, respectively. Hydrogen bonds are shown in green.

Table 1
Cases of *PDHA1* somatic mosaicism.

Patient	Sex	Clinical Phenotype	Lactic Acidemia	PDC enzyme SF (%)§	PDC enzyme SM (%)§	PDC enzyme lymphocytes (%)§	PDHA1 mutation	Samples evaluated for *PDHA1* mutation
Seyda et al. [8]	M	Neonatal lactic acidosis (neonatal lethality)	Severe	25–30	NA	NA	c.422G > T (p.R141L)	SF, liver, muscle
Boichard et al. [9]	M	Failure to thrive and axial hypotonia	Mild	62	NA	67	c.483C > T (p.Y161Y) del exon 5	Blood, hair
Mine et al. [10]	M	MRI findings: cortical atrophy and ventricular enlargement	Mild	55	NA	NA	c.787C > T (p.R263X)	Blood, SF, hair
Okajima et al. [11]	M	Delayed motor and cognitive development MRI findings: normal	Mild	33–53	37	68	c.592G > A (c.511_603del) del exon 6	Blood, SF, hair, buccal swab
Soares-Fernandez et al. [12] and Quintana et al. [7]	M	Dysmorphic features MRI findings: colpocephaly, corpus callosum dysgenesis, increased diffusion in the white matter, and bilateral subependymal cysts.	Significant	169	28	NA	c.904C > T (p.R302C)	Blood, SF, muscle
Ridout et al. [14]	F	Able to walk and reasonable comprehension with limited speech MRI findings: ventricular dilatation, sulcal widening, and relative course gyral pattern	Mild	51*	NA	NA	c.900-1G > A (p.S300Rfs*32)	SF
Coughlin et al. [13]	M	Failure to thrive and developmental delay MRI findings: periventricular white matter volume loss, with *ex vacuo* dilatation of lateral and third ventricules, thinned corpus callosum, and slightly small brainstem	Significant	148	24,45	NA	c.679 T > C (p.Y227H)	Blood, SF, muscle
Current proband	M	Seizures, failure to thrive and developmental delay MRI findings: hypoplastic corpus callosum, ventriculomegaly, volume loss of the brain parenchyma with marked prominence of the cortical sulci, and vermian hypoplasia	Significant	26,31	NA	NA	c.523G > A (p.A175T)	Blood, SF, buccal swab

SF, skin fibroblasts; SM, smooth muscle; NA, not available.
* Ridout et al. reported an overall PDC activity of 0.46 nmol/min/mg protein (normal range 0.7–1.1) [14].
§ PDC activity is the percent of the mean for the respective diagnostic laboratories.

PCR and Sanger sequencing of all exons and intron/exon boundaries of the *PDHA1* gene were performed on genomic DNA from cultured SFs, peripheral blood and buccal mucosa. The patient was mosaic for a novel, missense mutation, c.523G > A (p.A175T) (Fig. 1B, NM_000284.3). c.523G > A results in a substitution of a highly conserved alanine to threonine at position 175 of E1α (Fig. 1C). Different ratios of mutant adenine (A) and wild-type guanine (G) alleles were observed in the fibroblasts, peripheral blood and buccal cells, indicating a different mutant allele burden among different tissues (Fig. 1B). *In silico* prediction of structural changes of the alanine to threonine at 175 showed a potential disturbance of protein structure that may affect its overall interaction with E1β (Fig. 1C). Sequencing of the *PDHA1* gene from the peripheral blood and buccal cells of the proband's mother did not reveal any mutation, indicating a *de novo* event in this patient.

4. Discussion

Two-thirds of patients with functional PDC deficiency have a mutation in *PDHA1* [2–7]. *PDHA1* mosaicism was reported in six males [7–13] and one female patient [14] with different types of mutations (see Table 1 for details). Mosaic mutations may attenuate the clinical phenotype, as observed in surviving males with exonic mutations that resulted in skipping of exons 5 and 6 [9,11]. Complete exon skipping in hemizygous males is expected not to be compatible with survival. A mouse model of *Pdha1* exon 8 deletion demonstrated an embryonic lethality in male mice [15]. Mosaic point mutations can present with a milder phenotype if the mutant allele burden is low [10,11,13]. In contrast, mosaicism with a greater mutant allele burden (75%) in liver, SFs and muscle resulted in a male with a severe neonatal presentation leading to infant death [8]. The approximate mutant allele burden of our patient with severe clinical presentation was 70% in SFs, with lower burden in blood or buccal cells. The neurological severity is likely related to the mutational prevalence in brain cells.

A heterozygous p.A175P mutation was reported in a female presenting with mild lactic acidosis, decreased PDC activity in cultured SFs, neuronal loss, marked developmental delay, and poor head growth and weight gain [16]. In females, the phenotype is affected by X-inactivation.

The alanine-175 residue is within the α-helix containing the heterodimer interface of the E1α subunit (Fig. 1C) [17]. Several missense mutations causing PDC deficiency are located between exons 5 and 8 where regions of TPP-binding and intermolecular interactions reside [17]. The A175P substitution may cause conformational constraint of the protein backbone [16]. For this p.A175P variant, the elevated lactate with PDC and PDH E1 activities of 51% and 46% of the mean, respectively, in cultured SFs, along with this female patient's phenotype, are consistent with PDC deficiency [16]. The A175T substitution may cause altered hydrogen bonding patterns which alters E1α backbone and impede its interaction with E1β (Fig. 1C) [17]. By *in silico* structural prediction, the hydroxyl group on threonine forms a hydrogen bond with the carboxyl group of the polypeptide backbone of proline-172, which is a hetero-dimerization interface with E1β [18,19]. A p.P172L mutation causing severe PDC deficiency and cerebral abnormalities, encephalopathy, microcephaly, convulsions and severe psychomotor retardation in a female patient was reported [7]. Given the close proximity of alanine-175 to proline-172, p.A175T may alter the integrity of hetero-dimerization and cause significant functional PDC deficiency.

In conclusion, a novel mosaic mutation in *PDHA1* in a severely affected male patient is reported, expanding the mutation spectrum of mosaicism in *PDHA1*.

Compliance with ethics guidelines

All authors have reviewed and approved the manuscript for submission to be peer-reviewed.

Conflict of interest

Kristin K. Deeb, Jirair K. Bedoyan, Raymond Wang, Leighann Sremba, Molly C. Schroeder, George J. Grahame, Monica Boyer, Shawn E. McCandless, Douglas S. Kerr, and Shulin Zhang declare that they have no conflict of interest.

Informed consent

This investigation and consent process were approved by the Institutional Review Board of University Hospitals Case Medical Center.

Author Contributions

All authors contributed to the planning (KKD, JKB, DSK, SZ), conducting (KKD, MCS, GJG), and reporting (KKD, JKB, RW, LS, MB, SEM, DSK, SZ) of the work described in this article.

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