



Pitfalls of relying on genetic testing only to diagnose inherited metabolic disorders in non-western populations - 5 cases of pyruvate dehydrogenase deficiency from South Africa



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ABSTRACT

Pyruvate dehydrogenase complex (PDHC) deficiencies are a group of mainly infantile onset disorders stemming from defects in pyruvate catabolism. They are characterised by severe lactic acidosis and progressive neurodegeneration.

Although the *PDHA1* gene is implicated in most cases of PDHC deficiency worldwide, no pathogenic variants have been reported in South African patients to date, despite availability of *PDHA1* sequencing in the state diagnostic setting.

Methods: DNA from five patients with low to absent PDHC activity in fibroblasts were subjected to PDHC deficiency gene panel analysis. Included in the panel were: *PDHA1*, *PDHB*, *DLAT*, *DLD*, *PDHX*, *BOLA3*, *GLRX5*, *IBA57*, *LIAS*, *LIPT1*, *LIPT2*, *NFU1*, *PDP1*, *PDP2*, *SLC19A2*, *SLC19A3*, *SLC25A19*, *SLC25A26*, *TPK1* and *FBXL4*.

Results: No pathogenic variants were identified in 4 out of 5 cases investigated. A homozygous frame-shift mutation was detected in the *BOLA3* gene in one patient, supporting a diagnosis of multiple mitochondrial dysfunction syndrome type 2.

Discussion: A single, novel, homozygous *BOLA3* frame-shift mutation was detected in a black South African child with severe neurodegenerative disease and very low to absent PDHC enzyme activity. This finding of a homozygous mutation in a patient from a non-consanguineous background may indicate a need for further investigation in clinically similar cases as well as heterozygous carrier rates in unaffected individuals from the same ethnic background.

The paucity of identifiable mutations in 4 out of 5 South African patients with confirmed PDHC deficiency highlights the dangers in relying on Western population based genetic panels for diagnosing rare metabolic disease in genetically understudied populations.

1. Introduction

Pyruvate dehydrogenase complex (PDHC) deficiencies are a group of genetic disorders that affect pyruvate catabolism. The PDHC is an essential enzyme complex required for the conversion of pyruvate to acetyl-CoA, the rate limiting (and only irreversible) step of the Krebs cycle in mitochondria. It is made up of three essential enzymes, namely E1, E2 and E3. Defects in the proper functioning of any of the three enzymes in this complex ultimately results in a pathological build-up of

lactate and patients therefore typically present in infancy with severe lactic acidosis and progressive neurodegeneration [1–3]. Lactate:pyruvate ratios tend to be low or normal [4] in these patients due to the simultaneous build-up of pyruvate, distinguishing PDHC deficiency from other mitochondrial disorders of energy metabolism where lactic acidosis is often seen in the context of a high mitochondrial redox potential and hence high lactate:pyruvate ratio [5].

Five genes encode the core subunits of the complex; however pathogenic variants in at least 20 genes can cause this disorder. The most

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common cause (in Western populations) are pathogenic variants in the X-linked *PDHA1* gene, encoding the pyruvate dehydrogenase E1 alpha subunit [6,7]. Pathogenic variants in the other 4 genes encoding the subunits of PDHC are also associated with PDHC deficiency, namely *PDHB* (encoding the E1beta subunit), *DLAT* and *DLD* (encoding the E2 and E3 enzymes, respectively), and *PDHX* (encoding the E3 binding protein). However, various other genes encoding regulatory proteins and proteins involved in co-factor biosynthesis have also been implicated in PDHC deficiency [8].

The National Health Laboratory Service (NHLS) Inherited Metabolic Disease (IMD) Laboratory, closely affiliated with the University of Cape Town Division of Chemical Pathology, offered testing for PDHC activity for several years prior to 2012. The methodology used relied on assessment of PDHC activation by dichloroacetate (DCA) in fibroblasts, in which the $^{14}\text{CO}_2$ released by PDHC is trapped and measured. Non-specific decarboxylases in crude cell extracts can also act on [^{14}C] pyruvate to produce $^{14}\text{CO}_2$, thereby increasing the background radioactivity in an assay. To improve specificity therefore, one can use the extent to which pyruvate decarboxylase activity is increased by DCA, an activator of the specific form of pyruvate decarboxylase in which we are interested, namely PDHC. The normal enzyme is maximally stimulated about 50% by this compound, whereas the non-specific decarboxylases are unaffected by it. Thus, a profound deficiency in the specific enzyme would yield a % stimulation in crude cell extracts approaching zero [9].

This enzyme service of the NHLS was eventually withdrawn owing to loss of infrastructure, expertise and a move away from radioactive work, resulting in a disproportionate reliance on available *PDHA1* gene sequencing as the only remaining option for diagnosing PDHC deficiency in the South African state sector. However, despite this gene being the most commonly implicated cause of PDHC deficiency worldwide, no pathogenic *PDHA1* variants have been identified in any South African patients to date. The reason for this anomaly remained unknown.

Here we describe the findings in a pilot study looking at the genetics underlying PDHC deficiency in a handful of enzymatically confirmed South African patients, suggesting that this population group has a distinct spectrum of genetic aetiology in PDHC deficiency.

2. Methods

Ethical approval for this study was obtained from the UCT Human Research Ethics committee (HREC/REF 897/2016).

DNA samples from five patients previously confirmed through the NHLS IMD diagnostic service to have very low to absent PDHC activity in fibroblasts were retrieved from the NHLS/UCT metabolic disease DNA repository and forwarded to the NHS Oxford Genetics Laboratories for PDHC deficiency gene panel analysis. Consent was previously obtained for genetic investigations relating to PDHC deficiency in all these cases. Genes included in the panel were: *PDHA1*, *PDHB*, *DLAT*, *DLD*, *PDHX*, *BOLA3*, *GLRX5*, *IBA57*, *LIAS*, *LIPT1*, *LIPT2*, *NFU1*, *PDP1*, *PDP2*, *SLC19A2*, *SLC19A3*, *SLC25A19*, *SLC25A26*, *TPK1* and *FBXL4*.

Next generation sequencing of the coding regions and exon-intron boundaries of these genes was undertaken using custom HaloPlex (Agilent Technologies, Santa Clara, CA, USA) targeted library preparation followed by massively parallel sequencing using MiSeq (Illumina, San Diego, CA, USA). Putative pathogenic variants were confirmed by Sanger sequencing using ABI3730 (Applied Biosystems, Foster City, CA, USA).

Variants in the *BOLA3* gene were annotated in accordance with HGVS sequence variant nomenclature recommendations [10] against NCBI GenBank Reference Sequence NM_212552.3, and parents were tested for carrier status using ABI3500 (Applied Biosystems, Foster City, CA, USA).

3. Results

No pathogenic variants could be identified in 4 out of the 5 cases investigated, despite very low to absent enzyme activity in all. Limited available clinical and biochemical data from these 4 historic cases are depicted in Supplementary Table 1.

A novel, homozygous variant, c.159dupT p.(Asp54*), was detected in the *BOLA3* gene in one patient. This nucleotide change results in a frameshift, introducing a premature stop codon immediately downstream, and is therefore predicted to be pathogenic. The variant was confirmed by Sanger sequencing and both parents, though unrelated, were confirmed to be carriers, supporting a diagnosis of multiple mitochondrial dysfunction syndrome type 2 (MMDS2, OMIM#614299), rather than isolated PDHC deficiency as was initially suspected.

4. Case description

The MMDS2 patient identified here was a black South African child who presented to the NHLS/University of Witwatersrand genetics service at the age of 12 months with severe neurodegenerative disease. He exhibited normal development until about the age of 7 months, followed by sudden regression of milestones, floppiness, encephalopathy, increased tone and reflexes and cortical blindness. Brain CT scans done at the time indicated a demyelinating disorder. Biochemical analyses showed lactatemia with a markedly raised lactate/pyruvate ratio (32, patient range ≤ 20), atypical of isolated PDHC deficiency. PDHC activity was nonetheless significantly impaired in fibroblasts (-0.02 -fold stimulation of PDH activity by dichloroacetate compared to 1.47- and 1.54-fold stimulation in controls), while urine organic and amino acid analysis showed persistent ketosis and modestly elevated glycine levels, although the latter was never followed up in serum and csf prior to demise. An initial diagnosis of PDHC deficiency was made. He died at the age of 17 months. No consanguinity had been reported in the family.

5. Conclusion

A single, novel, homozygous *BOLA3* frameshift variant was detected in a black South African child with severe neurodegenerative disease and very low to absent PDHC enzyme activity (no PDHC activation on DCA stimulation).

Multiple mitochondrial dysfunctions syndrome type 2 (OMIM:614299), due to pathogenic variants in the *BOLA3* gene, is an extremely rare metabolic disorder worldwide with only seven pathogenic variants described in fifteen clinical cases [11–16]. It is essentially an iron-sulphur cluster biosynthetic defect, resulting in deficiencies of multiple lipoic acid cofactor-dependent enzymes (including PDHC) as well as in mitochondrial complexes I and II.

This finding of a novel homozygous *BOLA3* pathogenic variant in a patient from a non-consanguineous background may indicate a need for further investigation in clinically similar cases from the same ethnic background. It is not implausible that other cases are being missed in a country where most healthcare emphasis and resources are directed towards the heavy burden of infectious disease. Further investigations into the carrier frequency of this variant in the general black South African population may help to determine whether this is a likely cause of lactic acidosis in this population group.

The inability to genetically identify pathogenic variants in 4 out of 5 South African patients with confirmed PDHC deficiency highlights the dangers of relying on Western population-based genetic panels for diagnosing rare metabolic disease in genetically understudied population groups in the absence of enzymology. South Africa is home to a diverse population with genetically unique subpopulation groups, some of which are rich in genetic founder variants, likely introduced as a direct result of the country's unique immigration and migration history and cultural norms. Examples of these disorders include variegate

porphyria [17], glutaric aciduria type 1 [18], familial hypercholesterolemia [19,20], cystinosis [21], galactosemia [22], osteogenesis imperfecta [23,24], isovaleric acidemia [25] and MPV17 neurohepatopathy [26].

We propose that there may well be currently undescribed genetic defects underlying the PDHC deficiency seen in Southern African patients, which may not be readily detectable with current routine diagnostic panels. Comprehensive genomic investigations, such as whole genome or whole exome sequencing, as well as deletion/duplication screening and RNA studies in local patients may shed more light on this phenomenon. More in-depth biochemical investigations such as western blot analysis and co-factor levels, although not possible for these historic cases, should be looked at in future cases.

We further caution against trading valuable biochemical investigations for apparently comprehensive genetic studies in genetically understudied population groups without careful consideration and prior validation studies.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymgmr.2020.100629>.

Details of ethics approval

Ethical approval for this study was obtained from the UCT Human Research Ethics committee (HREC/REF:897/2016).

Patient consent statement

All patients consented to genetic testing for PDHC deficiency. Additional consent was obtained from the family of the patient described for publication of individual findings.

Details of funding

National Health Laboratory Services Research Trust, South Africa.

Declaration of Competing Interest

The authors have nothing to declare.

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