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Difficulties in recognition of pyruvate dehydrogenase complex deficiency on the basis of clinical and biochemical features. The role of next-generation sequencing

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ABSTRACT

Pyruvate dehydrogenase complex (PDHc) defect is a well-known cause of mitochondrial disorders (MD) with at least six responsible genes (*PDHA1*, *PDHB*, *DLAT*, *DLD*, *PDHX*, *PDP1*). The aim of this work was to assess the diagnostic value of biochemical methods in recognition of PDHc defect in Polish patients with suspicion of MD. In the first step, Western blot of the E1α subunit was performed on 86 archive muscle bioptates with suspicion of MD. In the second step, Sanger *PDHA1* sequencing was performed in 21 cases with low E1α expression. In the third step, 7 patients with negative results of *PDHA1* sequencing were subjected to whole-exome sequencing (WES). This protocol revealed 4 patients with *PDHA1* and one with *DLD* mutations. Four additional probands were diagnosed outside the protocol (WES or Sanger sequencing).

The molecular characterization of PDHc defect was conducted in a total of 9 probands: 5 according to and 4 off the protocol. Additionally, two affected relatives were recognized by a family study. Altogether we identified seven different *PDHA1* changes, including two novel variants [c.464T > C (p.Met155Thr) and c.856_859dupACTT (p.Arg288Leufs*10)] and one *DLD* variant.

The lactate response to glucose load in the *PDHA1* subset was compared to a subset of non PDHc-related MD. Opposite responses were observed, with an increase of 23% and decrease of 27%, respectively.

The results show that determining lactate response to glucose load and muscle E1α expression may contribute to distinguishing PDHc-related and other MD, however, WES is becoming the method of choice for MD diagnostics.

1. Introduction

Pyruvate dehydrogenase complex (PDHc) deficiency is a frequent cause of mitochondrial disorders. Progressive neurological symptoms usually start in infancy, but may be evident at birth or in later childhood, and adult onset is very rare. They may include developmental delay, brain malformations, microcephaly, poor muscle tone, seizures, intermittent ataxia, West syndrome, and Leigh-like syndrome.

There are six forms of PDHc deficiency, depending on the genetic background and damaged subunit of the enzyme complex. A few cases are known to result from mutations in genes encoding subunits: E1β (*PDHB*), E2 (*DLAT*), E3 (*DLD*), and E3BP (*PDHX*) or PDH phosphatase (*PDP1*). The most common causes are mutations in X-linked *PDHA1*, encoding the E1α subunit [1]. *PDHA1* maps to the Xp22.1 region and consists of eleven exons. The majority of mutations in this gene occur *de novo*. Hemizygous males are generally symptomatic, whereas heterozygous females present variable expression of the mutant and normal genes in different tissues as a result of the X-inactivation pattern [2].

This is the first genetic study of PDHc deficiency in Poland and we report novel pathogenic variants and recurrent causal mutations in the genes *PDHA1* and *DLD*. Our aim was also to check the utility of Western

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Table 1
Clinical, biochemical and molecular data of 11 patients with pathogenic variants in PDHc related genes.

Data and symptom(s)	Patient KI (p)	Patient KW (b)	Patient SzO (p)	Patient BF (p)	Patient KBS (m)	Patient PM (p)	Patient GP (p)	Patient KG (p)	Patient PM (p)	Patient ZJ (p)	Patient PZ (p)
Patient no.	1	2	3	4	5	6	7	8	9	10	11
Family no.	1		2	3		4	5	6	7	8	9
Sex	M	M	F	M	F	M	F	F	F	F	F
Age of onset	2 y	ND	Neonatal	Neonatal	2 y	15 m	3 m	7 m	Birth	4 m	1.5 y
Age at diagnosis	8.5	ND	2	4 y	ND	8 y	2.5 y	4.5 y	25 y	2 y	9 y
Neurological findings											
Psychomotor retardation/developmental delay	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Developmental delay (>3 y)	No	No		Yes	No	ND	Yes	Yes	Yes	Yes	Yes
Dysarthria	No	No	Nd	Yes	Yes	Yes	ND	ND	ND	ND	Yes
Microcephaly	No	ND	No	ND	No	No	Yes	Yes	Yes	Yes	No
Seizures	No	ND	No	No	No	No	Yes	Yes	No	Yes	No
Ataxia	No	ND	Yes	Yes	Yes	Yes	ND	No	ND	No	No
Hypotonia/hypertonia	No	ND	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Peripheral neuropathy	Yes	ND	ND	ND	Yes	Yes	ND	ND	ND	ND	No
Brain malformations											
Brain atrophy	ND	ND	No	Yes	No	ND	Yes	ND	Yes	Yes	Yes
Corpus callosum hypoplasia	ND	ND	No	ND	No	ND	Yes	Yes	ND	Yes	Yes
Cerebellum atrophy	ND	ND	No	No	No	No	ND	No	ND	VH	VH
Demyelinisation	ND	ND	No	Yes	No	ND	ND	ND	ND	ND	Yes
Basal ganglia abnormalities	ND	ND	Yes	ND	No	ND	ND	ND	ND	ND	Yes
Brain stem involvement	ND	ND	Yes	ND	No	ND	ND	ND	ND	ND	ND
Ocular findings											
Nystagmus	No	No	Yes	No	No	No	ND	No	Yes	No	No
Ptosis	No	No	No	No	No	No	ND	No	Yes	No	Yes
Oculomotor apraxia	No	No	Yes	No	No	Yes	ND	No	Yes	No	No
Biochemical findings											
Blood lactate (fasting) [mg/dL]	42.66	NA	26	20.8	47.4	62	36.2–65.6	39	42	54	104
Blood pyruvate (fasting) [mg/dL]	2.2	NA	2	1.7	2.3	3.7	NA	2.8	NA	NA	NA
Blood lactate (after carbohydrate) [mg/dL]	39	NA	25	29.6	NA	92	NA	NA	51	53	NA
Blood pyruvate (after carbohydrate) [mg/dL]	2.6	NA	1.5	2.1	NA	4.1	NA	NA	NA	4.5	NA
Blood lactate/pyruvate ratio	18	NA	13	12	20.7	17	NA	14	7.5	12	NA
CSF lactate [mg/dL]	40	NA	NA	NA	NA	67	75	47	87	NA	58.8
Alanine [μmol/dL]	NA	NA	559	180	NA	238	952	NA	222–1338	806	407
GC/MS urine	LA, PA, 2-KGA	NA	NA	2-KGA, LA	NA	2-KGA, LA	LA, 2-KGA	NA	2-KGA, LA	2-KGA, LA, MMA	Normal

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