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Pyruvate dehydrogenase deficiency in a female due to a 4 base pair deletion in exon 10 of the E1α gene

Willy Lissens*, Isabelle Desguerre[1], Chantal Benelli[2], Cécile Marsac[3], Françoise Fouque[2], Charles Haenggeli[4], Gérard Ponsot[1], Sara Seneca, Inge Liebaers and Linda De Meirleir

Department of Medical Genetics, University Hospital, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium, [1]Department of Neuropediatrics, Hôpital Saint Vincent de Paul, [2]INSERM U30, Hôpital Necker and [3]INSERM U75, Faculté Necker, 75015 Paris, France and [4]Department of Pediatrics, Hôpital Cantonal Universitaire, Geneva, Switzerland

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The pyruvate dehydrogenase complex (PDHc) is a multiprotein system that links glycolysis with the TCA cycle by oxidative decarboxylation of pyruvate to acetyl-CoA. It consists of three catalytic enzymes (E1, E2 and E3), a protein with an as yet unknown function (protein X) and two regulatory enzymes (PDH phosphatase and PDH kinase) monitoring the activation/inactivation of the complex by dephosphorylation/phos phorylation of the E1 component (1).

PDHc deficiencies have been associated with a wide spectrum of clinical presentations ranging from mild intermittent ataxia to severe neonatal lactic acidosis and early death (1–3). Although almost equal numbers of male and female patients with a PDHc deficiency have been described, most of the molecular defects have been localised in the X-linked E1α component of E1 (4).

We report on a 18-month-old dysmorphic female with a 4 base pair (bp) deletion in exon 10 of the Elα gene on one of her X-chromosomes. She presented with a severe metabolic acidosis: lactate and pyruvate concentrations in blood were 5.5 mM and 0.43 mM (L/P ratio 13:1), respectively, and 6.7 mM and 0.80 mM in cerebrospinal fluid (L/P ratio 8:1). She had complete agenesis of the corpus callosum, cortical atrophy, microgyria and was micro- and hydrocephalic. She developed early onset quadriplegia and myoclonic epilepsy. PDHc activity was measured in mononuclear cells, fibroblasts and muscle mitochondria and was 19%, 31% and 17%, respectively, of normal control values (5). Oxidation of pyruvate by intact mitochondria isolated from the patient's muscle was found to be very low (45%) compared to control values. In contrast, glutamate and succinate were normally oxidized. All muscle respiratory complexes, measured by spectrophotometry, were found to be normal. Western blot analysis of the patient's cultured fibroblasts showed normal PDH subunit immunoreactivity (data not shown). The whole coding region of the E1α gene was studied by SSCP analysis of six overlapping PCR fragments (W.Lissens *et al.*, submitted) and showed one abnormally migrating fragment. This fragment of 248 bp, defined by primers PDS3 (AGTGGATGGAA-TGGATATCC; cDNA nucleotide positions 864–883 (4)) and PDS4 (TTTAGTTCTTCCACACTGGC; cDNA nucleotide positions 1112–1093), and spanning exon 8, exon 9 and part of exon 10, was reamplified by PCR and directly sequenced in both directions. A mutation was localised close to primer PDS4 in exon 10, and for confirmation, exon 10 was reamplified from genomic muscle DNA using intron 9 primer PDH10 (TTTCATCACGCCGTCCTTGC) and intron 10 primer PDH70 (AACTTTTAAAGCCAACCTTC). Part of the sequence of this 226 bp fragment at the position of the mutation is shown in Figure 1. A normal E1α sequence was found in either direction, but starting from T at nucleotide position 1041 a second sequence was superimposed on the normal one. Close inspection indicated that the second sequence is an out of phase E1α sequence lacking 4 bp from nucleotides AAGTAAG at cDNA positions 1038–1044, resulting in AAG. Due to the direct repeat AAG around the central T, it was not possible to determine the exact position of the deletion since the removal

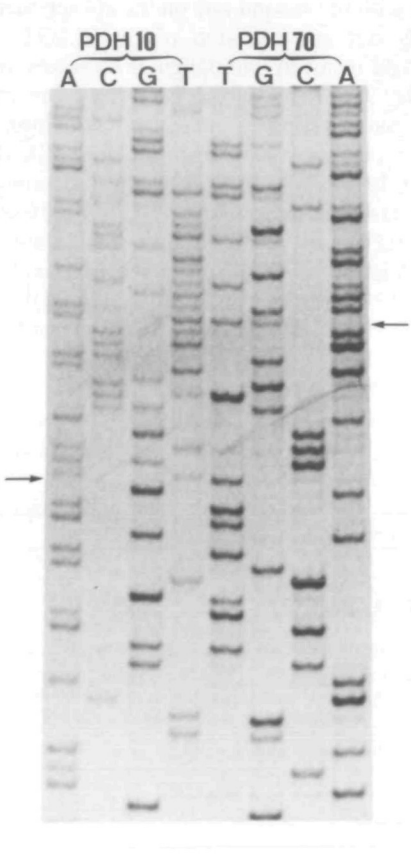

Figure 1. Direct sequence analysis of PCR-amplified exon 10 of the E1α gene of the patient in sense (primer PDH10) and antisense (primer PDH70) direction, using the Sequenase kit version 2.0 (USB). The arrows indicate the start of the superposition of a 4 bp deleted sequence on the normal one. The T at this position corresponds to bp 1041 in the normal E1α cDNA.

*To whom correspondence should be addressed

of four consecutive bases from this sequence will always result in AAG.

Our patient is thus a heterozygous carrier of a normal and a 4 bp deleted E1α gene. Inspection of the SSCP and cDNA sequencing gels indicated that in muscle the two alleles are expressed at a 35%/65% proportion (data not shown). The 4 bp deletion predicts a reading frame shift in the E1α polypeptide after amino acid 311 and a premature stop codon at position 324. The mutation was not found in the mother's genomic white blood cell DNA by sequencing of the PDH10/PDH70 PCR fragment. The father was not available for study.

In our patient, the 4 bp deletion is found in a 9 bp 2 bp overlapping, direct repeat sequence (GAAGTAAGAAGTA-AGA; underline: start of each 9 bp repeat; the T in bold corresponds to nucleotide position 1041), in which four AAG repeats are present. In a study by Krawczak and Cooper (6) of small gene deletions in human genetic disease, direct repeats are a common feature of all but one of 60 deletions analysed. Their results also indicate that small deletions are more compatible with a model of mutagenesis based on slipping mispairing during replication than with recombination or repair of DNA. The 4 bp deletion in our patient could easily be explained by the modified slipped mispairing hypothesis they propose: during replication, slipping mispairing of the fourth AAG repeat with the second one on the complementary strand, with looping out and excision of the AAGT sequence in between, would result in two daughter duplexes, one of which lacks the third AAG repeat and the T between repeats 3 and 4. Two alternative slipped mispairing structures could also explain the excision of 4 bp from the AAGTAAG sequence, but would be less stable than the former one since homology of only two bases (looping of AGTA) or one base (looping of GTAA) would be involved in loop formation. The fourth possibility, deletion of TAAG, could not be explained by this hypothesis. We therefore propose to refer to the mutation in our patient as 1038del4 (7). It is also noteworthy to mention that the 9 bp repeat sequence contains, albeit in opposite orientation, a 6 bp sequence (AGAAGT; cDNA nucleotide positions 1036–1041) that perfectly matches with a consensus sequence recognised as a hotspot for deletion (6). So far, three other females have been described with small deletions in the 9 bp repeat (8–10) and all of these, including our patient, have a 'cerebral' form of PDH deficiency (11,12).

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