Diagnosis of pyridoxine dependent epilepsy by urine tandem mass spectrometry screening

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Introduction & Method
• Pyridoxine dependent epilepsy (PDE), also known as antiquitin deficiency, is a recessive disorder of lysine catabolism caused by mutations in the ALDH7A1 gene. It causes an epileptic encephalopathy.
• α-aminoacidic semialdehyde (AASA) and its cyclic counterpart Δ1-piperideine-6-carboxylic acid (P6C) are increased due to deficiency of AASA dehydrogenase.
• PDE is responsive to pyridoxine treatment and can be characterised as “classical”, presenting in the neonatal period and an “atypical”, late onset presentation.
• Recently, two additional markers of PDE have been described, 6-oxo-pipeolic (6oxoPIP) and 6-(2-oxopropyl)piperideine-2-carboxylic acid (2OPP). We describe our experience with these markers during the diagnosis of a new patient with PDE and compare their diagnostic performance during retrospective analysis of stored samples from confirmed patients.
• P6C was analysed in positive ion mode as the butyl ester (184.1>82 m/z) using electrospray ionisation tandem mass spectrometry (MS/MS) as part of the urine metabolic screening (UMS). I
• P6C, 6oxoPIP (200.1>98 m/z) and 2OPP (242.2>184 m/z) were analysed as butyl esters and quantitated by LC-MS/MS.

Patient & Results
• Our patient presented at 4 months of age with seizures, which did not respond to routine antiepileptic therapy. Patient’s growth and development prior to presentation had been normal.
• UMS detected increased levels of P6C and its dimer form. Subsequent LC-MS/MS analysis confirmed the P6C increased and also showed increased 6oxoPIP and 2OPP (Fig 1).
• Two pathogenic compound heterozygous variants in ALDH7A1 were identified by singleton whole exome sequencing.
• The patient’s seizures resolved after being started on pyridoxine and continue to be seizure-free on “triple therapy” of a lysine restricted-diet alongside pyridoxine and arginine supplementation.
• Retrospective LC-MS/MS analysis of 9 samples from 8 known PDE patients showed increased P6C, 6oxoPIP and 2OPP.

Discussion & Conclusion
• Our patient (A) screened positive for PDE from a pre-treated sample. The biochemical markers remained increased post-treatment and also in another patient (B) undergoing treatment, indicating they are effective in detecting PDE patients even if pyridoxine treatment is commenced empirically.
• Retrospective analysis of samples from 8 other PDE patients (B, C, D, E, F, G, H and I) stored for up to 15 years -80 to -30°C also revealed significantly increased 6oxoPIP and 2OPP, confirming the diagnostic utility and stability of these biomarkers.
• At least 2 of the 3 biomarkers were increased in all PDE patient samples.

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Figure 1 Graphs (log scale) show P6C (arb units/mmol creatinine), 6oxoPIP (µmol/mmol creatinine) and 2OPP (arb units/mmol creatinine) in urine samples. P6C, 6oxoPIP and 2OPP have good specificity as biomarkers for PDE compared to controls (n = 59). * Indicates patient is being treated