ePOO8

Cystathionine beta synthase deficiency patients are being missed by newborn screening

Kimberly Chapman[1], Danae Bartke[2], Margie McGlynn[2], Harvey Levy[3], Can Ficicioglu[4]

[1]*Children's National Hospital;* [2]*HCU Network America;* [3]*Boston Children's Hospital;* [4]*The Children's Hospital of Philadelphia*

Introduction: Newborn screening for Cystathionine beta Synthase (CBS deficiency, also known as Homocystinuria) was initiated in some states as early as 1968 with the aim of preventing devastating complications from inadequate early treatment. During the 1990s, improvements in technology allowed for CBS deficiency to be detected by mass spectrometry detection of methionine levels from the dried blood spot card. Not until 2009 was CBS deficiency recommended to be universally screened in all US states following its inclusion in the Recommended Uniform Screening Panel (RUSP). Due to the organization of newborn screening laboratories by state, there is not uniformity in cutoffs across states. More recently, some states have started using second-tier screening, where they lower the methionine cutoff, and methionine/phenylalanine ratio and measure total homocysteine for those above the cutoff to decrease false positives.
Methods: HCU Network America, a family support group for individuals with homocystinurias including CBS deficiency, the cobalamin and remethylation disorders, asked its members whether they were being identified by newborn screening, as part of an IRB approved research protocol, after several families had come forward asking about how to obtain their newborn screening results since they thought they had been missed by newborn screening. Through the families, the date of initial newborn screen card collection, date of diagnosis, the state where the newborn screening was done, and, in some cases, the actual newborn screening reports were collected and analyzed.
Results: Twenty-three individuals were identified to have CBS deficiency whose newborn screening was not flagged positive. These 23 individuals had screening done in approximately 14 different states. Many of the state labs (of those from which we have data) had cutoffs of greater than or equal to 100 µmol/L methionine at the time of newborn screening, although a few had lower methionine cutoffs. Of those with CBS deficiency for whom we have data, many of the individuals had methionine levels in the 30s-60s µmol/L detected from their newborn screening cards.
Conclusion: Homocystinuria caused by CBS deficiency may not always be detected by newborn screening, especially with our current cutoffs. We recognize that we do not have a complete list of possible false negative CBS deficiency cases, and it unclear whether they are not being reported or whether they are not being diagnosed. Evidence from Europe indicates that up to 50% of cases may be missed with our current newborn screening approaches. Some of this is a consequence of the lower methionine levels seen in individuals with pyridoxine-responsive CBS deficiency, however the cost of missing a diagnosis is significant. Thus, state labs are encouraged to consider a lower methionine cutoff, along with adding methionine/phenylalanine ratio, and to add a second-tier screen for total homocysteine for those who exceed the threshold. Also, clinicians should have a high level of suspicion and should do total homocysteine screening in patients who present with hypermobility, ectopic lentis, tall stature, learning differences/intellectual disability and/or thrombosis. Moreover, awareness throughout the field of genetics that there is a possibility that newborn screening cannot always pick up all the disorders for which individuals are screened should motivate us to continue to collect cases and look for ways to improve screening for health and well-being of our patients.

https://doi.org/10.1016/j.gim.2022.01.046

ePOO9

Identification of a novel exonic deletion in the *GALNS* gene causing Morquio syndrome

Kathryn DeLong[1], Annette Feigenbaum[2], Laura Pollard[3], Andrew Lay[3], Timothy Wood[4]

[1]*Rady Children's Hospital San Diego;* [2]*University of California San Diego, Rady Children's Hospital San Diego;* [3]*Greenwood Genetics Center;* [4]*Children's Hospital of Colorado*

Background: Mucopolysaccharidosis (MPS) IVA or Morquio syndrome is a rare lysosomal storage disorder caused by N-acetylgalactosamine-6-sulfatase deficiency. The condition is characterized by intracellular accumulation of the glycosaminoglycans keratan sulfate and chondroitin-6-sulfate, which classically leads to progressive skeletal and joint abnormalities, short stature, cardiorespiratory compromise, impaired vision, hearing loss, and hepatomegaly. The diagnosis is made by the identification of reduced N-acetylgalactosamine-6-sulfatase activity as well as detection of compound heterozygous or homozygous pathogenic variants in *GALNS*.
Case presentation: We present a case of two sisters with the severe classic phenotype of Morquio syndrome who were born to healthy non-consanguineous parents of East Indian background. Patient 1 first presented at age 1.5 years with trouble walking and abnormal gait with a subsequent skeletal survey suggestive of dysostosis multiplex. She was diagnosed at age 2 years with Morquio syndrome based on reduced leukocyte N-acetylgalactosamine-6-sulfatase activity of 2.8 nmol/17hr/mg protein (reference range 45-443 nmol/17hr/mg protein). Medical history for patient 1 includes normal cognition, truncal shortening, scoliosis, distal joint laxity, cervical arthropathy, gibbus deformity, corneal clouding, and obstructive sleep apnea. Patient 2, the younger sister, was diagnosed with MPS IV at age 3 following a history of frequent falls and identification of genu valgum and early dysostosis. Given the family history, she was tested for Morquio syndrome and N-acetylgalactosamine-6-sulfatase leukocyte activity was <0.062nmol/17hr/mg protein (reference range 45-433 nmol/17hr/mg protein). Patient 2 has a history of cervical C1-C2 instability and occipitocervical stenosis, severe bilateral genu valgum, hip dysplasia, leg length discrepancy, pectus carinatum and corneal clouding. Both children, now at age 11 and 10 years respectively, need to use a wheelchair for any distance outside the home, despite orthopedic procedures.
Prior genetic testing for MPS IVA, which utilized next generation sequencing (NGS), did not identify sequence variants associated with MPS IVA, however it was noted that a large region of the *GALNS* gene corresponding to exon 9 was not covered in the sequencing data. After establishing care with our center, repeat enzyme testing identified absent N-acetylgalactosamine-6 sulfatase activity for patient 1 and significantly reduced activity of 0.18 nmol/17hr/mg protein for patient 2 (reference range 49-255 nmol/17hr/mg protein; affected range < 7 nmol/17hr/mg protein). Subsequent polymerase chain reaction (PCR) amplification of the coding exons for *GALNS* produced an appropriate product for all exons with the exception of exon 9 and we hypothesized that a deletion encompassing exon 9 was present in our patients. Therefore, an allele specific PCR assay was designed to confirm the exon 9 deletion and determine the precise deletion breakpoints (c.899-397_1003-18632del) for our patients. The reference mRNA sequence was NM_000512.4 with codon 1 corresponding to the start ATG.
Conclusion: To our knowledge, the pathogenic deletion identified in our patients (c.899-397_1003-18632del) has not been reported in the literature. Various deletions encompassing at least one exon have been reported in *GALNS,* however none encompassing only exon 9. NGS is increasingly utilized as a first-tier test for lysosomal storage diseases via targeted gene panels or exome/genome sequencing. The inability of this testing to detect large deletions or

rearrangements, especially in the heterozygous state, could lead to a delayed or even missed diagnosis. For example, if the deletion described in this case was *in cis* with missense change in *GALNS*, NGS would label the patient as a heterozygote rather than affected. Our case highlights the benefit of biochemical analysis for lysosomal storage disorders in cases with equivocal or incomplete molecular analysis. Molecular analysis is useful to confirm a biochemical diagnosis and assist with genetic counseling and future prenatal testing and carrier testing. Recognizing limitations of molecular testing is important to ensure accurate diagnosis and treatment for individuals with Morquio syndrome in a timely manner.

https://doi.org/10.1016/j.gim.2022.01.047

eP010

Conservative management with serial biochemical monitoring for newborn screen detected Maple Syrup Urine Disease (MSUD) patients without metabolic decompensation

Danielle DeMarzo-Burdsall[1], Yazmin Enchautegui Colon[1], Ashley Ethriedge[1], Michelle Peters[1], Chelsie Hollas[1], Anne Tsai[1]

[1]*University of Oklahoma Health Sciences Center*

Background: Maple syrup urine disease (MSUD) is a rare inborn error of metabolism characterized by deficiency of branched-chain alpha-keto acid dehydrogenase that metabolizes the three branched-chain amino acids (BCAAs) leucine, isoleucine, and valine. The result of this enzyme deficiency is a toxic buildup of metabolites, typically within the first 24-48 hours of life. The main neurotoxic effects are due to accumulation of large amounts of leucine which can lead to severe cerebral edema. Treatment requires immediate medical intervention to lower leucine levels. Various techniques have been used to reduce plasma leucine levels including invasive techniques such as dialysis and continuous renal replacement therapy. Risks are associated with such techniques including infections, hypovolemia, electrolyte disturbances, and fluid overload. These risks must be balanced with the risk of cerebral edema and neurologic side effect of metabolic intoxication from high leucine levels. Whatever the means, the goal remains rapid reduction in leucine levels.
Case presentation: We present two cases of patients with MSUD ascertained by abnormal newborn screens. Their initial leucine levels were critically elevated but showed no evidence of decompensation. They were both able to be successfully managed with conservative interventions to safely reduce leucine levels. Patient #1 is a 3-month-old male infant identified on newborn screen on the 5th day of life. Confirmatory testing drawn on the 6th day of life which revealed a Leucine level in the critical range of 2022 µmol/l. He was admitted to the hospital the same day for emergent management. The baby was doing well, gaining weight and neurologically intact. He was managed conservatively with a BCAA restricted formula, MSUD Anamix Early Years, intravenous fluids with D10, and NS to maintain Na greater than 137. We were able to safely reduce leucine levels by 39% within the first 24 hours and a 94% reduction within 72 hours, resulting in normalization of leucine levels to normal range of 109 µmol/l. At this time, it was indicated to add isoleucine and valine back into the diet, but the facility's pharmacy did not stock these amino acid supplements. Therefore, we used IVA Anamix Early Years formula, which is leucine free but contained our desired isoleucine and valine. This was utilized until we received shipment of isoleucine and valine supplements. On day 4 of admission, natural protein was able to be safely reintroduced and appropriately formulated branch amino acid supplement shipments were received. He was monitored with daily plasma amino acids for a total of 7 days to ensure stability before being discharged. He continues to be routinely followed in our clinic on a weekly basis and is doing well. He is thriving with a current weight of 7.1kg and length of 61.5cm. He was molecular testing confirmed to have biallelic pathogenic missense variants in trans in the *BCKDHB* gene (NM_183050.4). These changes were identified as c.410C>T (p.Ala137Val) and c.1A>T (p.Met1?). He has no obvious neurologic deficits.
Patient #2 is a 5-year-old boy also identified on newborn screen on the 4th day of life. Confirmatory labs draw on that same day revealed a leucine level of 1846 µmol/l. He had a similar course with similar results. He was managed conservatively with a leucine free diet and intravenous fluids. Also, in this case, there was a delay in shipment of supplemental isoleucine and valine, so natural protein was initiated earlier on the 3rd day of hospitalization due to a critically low valine level. While this may have slowed the rate of decline of leucine levels, we were still able to achieve a 32% reduction in leucine within the first 48 hours of hospitalization and stabilization for discharge was achieved within a week to a level of 167 µmol/l. He has since been able to receive a liver transplantation at one year of age and remains neurologically intact. Subsequent molecular testing has revealed that he carries a homozygous 213.22 kb deletion in BCKDHB gene which was consistent with the reported consanguinity of parents being second cousins.
Conclusion: Advances in newborn screening and early detection has modified the disease progression in patients with MSUD. When a patient is diagnosed before metabolic decompensation, without proteolysis, the reducing of leucine can be quickly and successfully achieved without invasive measures. The success of these interventions can be assessed by clinical presentation, CMP, and frequent monitoring of plasma amino acids. As demonstrated in our patients, daily reduction is 600-750 µmol/l vs historical patients admitted with symptoms, average dropping 300-350 µmol/l. Our two patients with MSUD demonstrated the evidence that, without metabolic crisis, conservative management of high leucine levels can result in safe and timely reduction of leucine with no apparent neurologic sequela. While each case must be considered individually, these cases may change our standard approach. It may at least present an alternative approach in circumstances where continuous renal replacement therapy or a form of dialysis may not be readily available for a neonatal patient provided levels can be readily monitored to ensure an adequate decline in levels.

https://doi.org/10.1016/j.gim.2022.01.048

eP011

Diagnosis of *DNAJC12*-deficient hyperphenylalaninemia offers targeted therapeutic options to counteract neurotransmitter deficiency

Colleen Donnelly[1], Jaya Ganesh[1], Emalyn Cork[1], Lissette Estrella[1], Ilona Ginevic[1], Casey Campbell[1], Jessica Sahota[1], Lauren Mason[1], Suzanne Miller[1]

[1]*Icahn School of Medicine at Mount Sinai*

Background: *DNAJC12*-related hyperphenylalaninemia is a recently described inborn error of metabolism (IEM) associated with hyperphenylalaninemia and neurotransmitter deficiency, caused by biallelic pathogenic variants in the *DNAJC12* gene. Clinical features include global developmental delay, intellectual disability, autism spectrum disorder, and dystonia. *DNAJC12*-encoded protein functions as a chaperone facilitating the proper folding of the biopterin-