ePOO8

Cystathionine beta synthase deficiency patients are being missed by newborn screening

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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.

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ePOO9

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

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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