Newborn screening for medium chain acyl-CoA dehydrogenase deficiency: Performance improvement by monitoring a new ratio

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A B S T R A C T

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is a fatty acid oxidation disorder included on newborn screening (NBS) panels in many regions that have expanded using tandem mass spectrometry for acylcarnitine screening. False positive (FP) screening results for MCAD deficiency have previously been linked to very low birth weight (VLBW) infants and those who are heterozygous for the common mutation, p.K324E. Previous studies have identified these causes of FP screens by sequencing residual dried blood spots. From our cohort of FP screens in Georgia, we identified an elevation at the same mass as octenoylcarnitine (C8:1) causing elevations of octanoylcarnitine (C8) not due to MCAD deficiency. We reviewed biochemical results from 2011 to 2013 for all newborn screens positive for MCAD deficiency in Georgia to identify screening criteria to allow these cases to be identified prospectively, thus saving families the stress of additional testing on their newborn and reducing healthcare costs while improving screening performance for the screening program. We identified the C8/C8:1 ratio as an effective marker, and developed criteria that will reduce FP screening results due to this interfering substance.

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1. Introduction

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency (OMIM # 607008) is the most common fatty acid oxidation disorder in the United States, and is included on newborn screening (NBS) panels in every state. Clinically, MCAD deficiency is characterized by hypoketotic hypoglycemia, and can present as sudden death as the result of a mild, intercurrent illness, when the body is not able to effectively mobilize fatty acids in response to fasting [1–3]. Treatment revolves around preventing these episodes of fasting with frequent feeds and prompt attention during illnesses that result in decreased dietary intake. The goal of NBS is to identify affected infants before they have an acute episode, implement appropriate dietary interventions, and provide them with protocols for emergency situations.

The conditions included in NBS panels are dictated by local laws. In the United States, the recommendations made in 2006 by the American College of Medical Genetics form the backbone of most states’ screening programs. MCAD deficiency was included as a primary target in this panel. While state laws dictate which conditions are included in NBS panels, state laboratories must decide how to perform the screening. Screening for MCAD deficiency is universally performed using tandem mass spectrometry (MS/MS) with octanoylcarnitine (C8) as the primary marker.

As expanded NBS panels have become adopted on a widespread basis, screening programs have observed that a disproportionately number of FP screens occur in very low birth weight (VLBW) infants who are often in the neonatal intensive care unit (NICU). Congenital hypothyroidism [4,5] and congenital adrenal hyperplasia [6–8] have been particularly troublesome conditions in the VLBW population, with some states adopting specific criteria for screening this cohort. FP results for MCAD deficiency have also been an issue among VLBW infants. In Ohio, retrospective sequencing of FP MCAD deficiency screens suggested that two situations were the most frequent causes for FP screens: individuals who were heterozygous for the common mutation, p.K324E, in ACADM, and premature infants. Based on further biochemical studies, the authors proposed that an interfering substance was present in these samples, causing elevations of C8 that were not due to MCAD deficiency [9]. This abnormal profile has been identified and discussed at the Region 4 Stork (R4S) training course for NBS interpretation [10].

Acylcarnitine profiles can be interpreted both qualitatively, based on the mass spectra, and quantitatively, using the calculated analyte...
concentrations. In NBS for MCAD deficiency, TP cases will have elevated C8 concentrations, and a characteristic pattern on qualitative observation with C8 being more prominently elevated than hexanoylcarnitine (C6) and decanoylcarnitine (C10). Not all NBS programs perform a qualitative review of profiles, with some, including Georgia, choosing to rely only on established cutoffs for selected markers. Qualitative review of acylcarnitine profiles from VLBW infants revealed the presence of an abnormal peak with the same mass as octenoylcarnitine (C8:1) much more frequently than in normal birth weight infants [10]. There is no known metabolic condition that results in an accumulation of C8:1. This abnormal species resulted in observed elevations of C8, which were not caused by MCAD deficiency. The most likely explanation for this secondary elevation of C8 is an isotope effect from the species that has a mass equal to C8:1. This finding was observed in NBS profiles from laboratories using both derivatized (butyl-esters) and non-derivatized preparation methods.

This paper sets out to describe quantitative values obtained from this interfering substance, and identify screening criteria to reduce FP results. The goal of the present study was to examine the results of NBS acylcarnitine analyses to determine if FP screens due to elevated C8:1 could be identified during the screening process before they were reported rather than being followed up for confirmatory testing. Recent studies have focused on the reduction of FP screens through the identification of novel exogenous interferences for isovaleric acidemia [11] and malonic aciduria [12]. There has also been significant effort towards performance improvement for newborn screening including second tier tests [13,14] and post-analytical tools that take advantage of a shared database of reference percentiles and true positive (TP) cases [15,16]. These alternative strategies could minimize testing and reduce worry and stress for families [17–19].

### 2. Materials and methods

In an attempt to identify a biochemical pattern that would allow us to distinguish certain FP screens before results are reported to healthcare providers, we extracted screening results from Emory University’s Newborn Screening Follow-up Database of all infants with a positive screen for MCAD deficiency between 2011 and 2013. We obtained birth weight, screening values for acylcarnitines and amino acids, case outcome, and the testing required to arrive at this outcome (repeat dried blood spot card for NBS, confirmatory biochemical testing, molecular genetic analysis). This study was classified as minimal risk and approved by the Institutional Review Board of the Georgia Department of Public Health (DPH). In Georgia, if the C8 concentration is between 0.35 and 0.67 nmol/mL and the C8/acyethylcarnitine (C2) ratio is ≥0.025, a screen is reported out as positive for MCAD deficiency. If the C8 concentration is >0.67 nmol/mL, the screen is reported out as a “critical high,” regardless of the C8/C2 ratio. Infants with “critical high” results for MCAD deficiency are typically referred for immediate confirmatory testing. Routine positive screens are evaluated and recommendations are made either for confirmatory testing or repeat NBS on a case by case basis. NBS follow-up for most conditions, including MCAD deficiency, is coordinated via a partnership between Emory University Department of Human Genetics and DPH. Final determinations on the status of a screening result are made by a team of nurses, clinical geneticists and laboratory geneticists. Testing is often coordinated through primary care providers and hospitals, but final outcomes are determined centrally.

Since the start of 2011, Georgia’s NBS program has recorded quantitative results for C8:1; however no cutoff was established, and this analyte has not been taken into consideration prior to reporting screening results. The goal of this project was to develop biochemical screening criteria that would allow the program to reduce FP screens in an effort to improve laboratory performance while also reducing healthcare costs and stress on families.

### 3. Results

Between 2011 and 2013, 132 screens were positive for MCAD deficiency according to the above criteria. This included 18 TP cases of MCAD deficiency. Expanded NBS was implemented in Georgia in 2007, and the incidence of MCAD deficiency between 2007 and 2012 is 1:15,172 (55 cases in 834,466 births), making it the most common disorder detected by MS/MS in Georgia. Of the remaining screens, 92 cases were classified as TP, 4 were determined to be carriers, and 18 expired before the abnormal NBS could be resolved. Further subdivision of the FP cohort showed 69 screens (67 infants) with a reported birth weight of <1500 g (selected as the cutoff for VLBW in this study) according to the definition of “very low birth weight” determined by DPH) and 23 had birth weights >1500 g. Over the study period, the positive predictive value (PPV) of MCAD deficiency screening was 13.6%.
All TP MCAD deficiency cases were initially reported out as “critical abnormalities,” and had confirmatory testing (plasma acylcarnitine and urine organic acids or acylglycines) recommended immediately, along with fasting precautions and instructions for families to report to emergency departments in case of poor dietary intake. This cohort included one affected infant with NBS findings strongly suggestive of MCAD deficiency who died before confirmatory testing could be performed. Follow-up of this individual case showed that the submitting hospital did not promptly ship the specimen to the screening lab. Ten of the normal birth weight (NBW) FP screens were critical abnormalities, as were 15 of the VLBW FP. The weight distribution of TP and FP cases is shown in Table 1. In the NBW FP cohort, 7 infants had their findings resolved by repeat NBS, while 16 had immediate confirmatory testing recommended. In the VLBW FP group, more infants had their cases resolved by repeat NBS (46) than confirmatory testing [21], due to a combination of lower abnormal analyte values, as well as a reluctance on the part of NICU teams to initiate further blood draws on these infants. Two of the VLBW FP cases had no further follow-up recommended, based on a combination of previously normal screens and mild biochemical abnormalities. The NBS cards of all VLBW infants indicated that they were in the NICU. There were no known cases of MCAD deficiency missed by NBS in Georgia during the study period. To our knowledge, none of the infants with FP screens have presented clinically with a disorder resembling MCAD deficiency after the NBS results were resolved.

4. Discussion

To improve the performance among VLBW infants, we decided to calculate the ratio between C8 and C8:1 (C8/C8:1) to identify cases where the interfering analyte was elevated, while not compromising the identification of FP cases of MCAD deficiency. A plot of C8/C8:1 against birth weight is shown in Fig. 1 for FP results in VLBW and NBW infants, as well as for TP cases of MCAD deficiency identified during the study period. This chart illustrates the distinct BW categories of our two FP cohorts. Tabular data showing the mean value and ranges of C8, C8:1, and the ratios (currently in use, and newly proposed) are included in Table 1. This also includes the reference percentiles for the analytes that were previously in use for screening in this condition. The VLBW FP infants have C8/C8:1 ratios that are lower than the TP cases identified over the same time period, and discrimination using this analyte was much better than C8 alone or other ratios identified for MCAD deficiency screening. A summary of p-values is shown in Table 2, with the comparisons between both FP groups (NBW and VLBW) and TP cases of MCAD deficiency for each analyte. All analytes and ratios (C8, C8:1, C8/C2 and C8/C8:1) were significantly different (p < 0.05) between both FP groups and TP cases of MCAD deficiency, with the exception of C8:1 between VLBW FP screens and TP cases of MCAD deficiency. Fig. 2 shows the concentration of C8 plotted against the newly developed ratio. The discrimination between VLBW FP screens and TP MCAD deficiency screens is clear, but there is overlap between the NBS FP screens and TP cases. The overlap with both groups would increase if we considered the worldwide population of MCAD deficiency cases represented in the R4S database, as participants have reported at least 366 cases of MCAD deficiency with a C8 concentration of <1.29 nmol/mL, the lowest concentration in a TP case identified by NBS in Georgia. Redefining an effective screening cutoff using only the existing analytes and ratios would not offer reduction in FP screens while still identifying all TP cases.

To further illustrate the improvements available with this additional ratio, we evaluated each of the screens in all three groups with the current version of the R4S single condition tool for MCAD deficiency. Profiles are assigned a score, and a given profile with a score greater than the 1st percentile of the known disease range for TP cases is considered informative [20]. All 18 TP cases of MCAD deficiency in Georgia had informative scores using the single condition tool. For the FP screens in NBW infants, 43.5% (10/23) had profiles that gave informative scores using the same tools. In the VLBW cohort, 53.7% (36/67) of the unique screens gave informative scores for MCAD deficiency. With the limited options available for performance improvement using the existing suite of options, we focused on the additional ratio as a screening tool, as we felt that simply increasing the cutoff for C8 could result in missed cases, and decreasing it would result in even more FP cases. The existing single condition tool offered some improvement, if uninformative profiles were not followed up on, but not to the extent possible when adding in the additional analyte and ratio. The discrimination between affected and unaffected babies in the NBW cohort was not as clear, but a reduction in FP cases is possible while still maintaining high sensitivity. Cutoff values for C8/C8:1 of 4.0 and 5.0 for infants with birth weights <1500 g and ≥1500 g respectively would have eliminated all but three FP screens (all with birth weight >1500 g). All TP cases of MCAD deficiency in GA during the study period would have been correctly identified using these criteria. Applied retrospectively, these screening criteria would have improved the PPV for MCAD deficiency in Georgia to 78.6%. To assist other NBS programs that may experience similar problems with FP screens due to this interference, results from this study were added to the R4S database and used to create a dual scatter plot for distinguishing this profile from TP cases of MCAD deficiency (available at www.clir-r4s.org). The utility of dual scatter plots in interpreting NBS results for very long chain-acyl-CoA dehydrogenase deficiency has been demonstrated recently [20,21]. The scatter plot is available to all registered users of the R4S website, and analytes have been selected for programs using derivatized and underivatized methods.
5. Conclusions

We have identified an additional analyte, C8:1, and ratio, C8/C8:1, that can be monitored by NBS laboratories within their existing acylcarnitine profiles to help reduce FP screens for MCADD deficiency. We have also developed criteria for the Georgia NBS laboratory to reduce FP screens for MCADD deficiency using an additional ratio as the secondary screening criteria in a cutoff based system. The combination of the R4S single condition tool, plus the dual scatter plot created in this study will allow other screening labs to identify this type of profile in their programs, if that is their preferred method of triaging screening results. This change in the screening algorithm for MCADD deficiency can be used to improve performance of NBS laboratories, as well as reduce healthcare costs and stress for families of infants with FP screens.

References