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Elevated methylmalonic acidemia (MMA) screening markers in Hispanic and preterm newborns

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Abstract

Analysis of California newborn screening (NBS) data revealed a high prevalence of Hispanic infants testing positive for methylmalonic acidemia (MMA), a trend seen for both true- and false-positive cases. Here we show that Hispanic infants have significantly higher levels of MMA screening markers than non-Hispanics. Preterm birth and increased birth weight were found to be associated with elevated MMA marker levels but could not entirely explain these differences. While the preterm birth rate was higher in Blacks than Hispanics, Black infants had on average the lowest MMA marker levels. Preterm birth was associated with lower birth weight and increased MMA marker levels suggesting that gestational age is the stronger predictive covariate compared to birth weight. These findings could help explain why MMA false-positive results are more likely in Hispanic than in Black infants, which could inform screening and diagnostic procedures for MMA and potentially other disorders in newborns.

Keywords

Newborn screening; Public Health; Inborn metabolic disorders; Genetics; Metabolism

1. Introduction

Methylmalonic acidemia (MMA), an inborn metabolic disorder associated with intermittent and often severe metabolic decompensation [1,2], is detected with newborn screening (NBS) by elevated propionylcarnitine (C3) and its ratio with acetylcarnitine (C3/C2)

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Author contributions

GP and CS designed the study, GP performed the statistical analysis, GME, TMC, HZ and CS provided input on data analysis and interpretation, and GP, CdF, TMC and CS wrote the manuscript, which all authors edited and approved.

Conflict of interest

The authors report no conflicts of interest in this work.

[3,4]. Although screening using tandem mass spectrometry (MS/MS) now identifies most newborns with MMA, it also creates a high number of false-positive results at a ratio of 5 infants without the disorder to 1 infant with the disorder. Avoiding false-positive results in NBS could reduce the emotional and financial burden of follow-up testing. Previously, an analysis of screening data reported by the California NBS program revealed a disproportionately high frequency of Hispanic newborns testing positive for MMA, a trend seen for both true- and false-positive cases [5]. While the birth prevalence for specific disorders is known to vary among different racial/ethnic groups [6,7], identifying a higher number of MMA false-positive cases with Hispanic ethnicity was unexpected. Here we assess the hypothesis that Hispanic newborns have inherently higher C3 or C3/C2 levels, which could directly increase the number of MMA false-positive cases in this group. Additionally, we investigate factors such as preterm birth and birth weight that could influence NBS results.

2. Material and methods

This study was approved by the Institutional Review Boards at Yale University (Protocol ID: 1505015917), Stanford University (Protocol ID: 30618) and the State of California Committee for the Protection of Human Subjects (Protocol ID: 13-05-1236). We analyzed analytic and demographic data from 10,000 healthy, screen-negative infants born between 2013 and 15 that were selected at random by the CA NBS program (Table 1). Data included metabolic analytes measured by tandem mass spectrometry [3], as well as race/ethnicity, gestational age (GA), birth weight (BW), age at blood collection, and total parenteral nutrition (TPN) status. Because TPN can artificially influence metabolite levels, the 289 newborns with positive or unknown TPN status were removed from analysis, as were 260 newborns with unknown race/ethnicity. The remaining 9451 infants were assigned to one of four race/ethnicity groups (Asian, Black, Hispanic or White) based on NBS program guidelines [6]. After removing 7 cases with missing GA data, 9444 were categorized as full term (GA≥37 weeks) or preterm (<37 weeks). Of the 9444 infants with known race/ethnicity and GA, 9417 had available BW and gender information. Differences in mean C3 and C3/C2 values between group pairs were evaluated using Cohen's d [8] and a *t*-test.

3. Results

Both C3 and C3/C2 were significantly higher in Hispanic (n=4685) than in non-Hispanic newborns (n=4766) (C3: Cohen's d=0.15, 95% CI 0.13 to 0.21; $P < .001$, and C3/C2: Cohen's d=0.30, 95% CI 0.26 to 0.34; $P < .001$). Pairwise comparisons between individual groups showed that Hispanics had higher C3 than Blacks or Whites, and higher C3/C2 than each of the other groups (Fig. 1A–B, Table 2). On average, C3 and C3/C2 were lowest among Blacks. C3 levels were generally higher in males than females (Hispanics, Whites and Asians), while C3/C2 was higher in females (Hispanics, Whites and Blacks).

To evaluate the effect of gestational age, we compared preterm versus full-term analyte levels within each group and found higher C3 and C3/C2 among preterm infants for Hispanics, Whites and Asians, but not for Blacks (Fig. 1C–D, Table 2). In contrast, the preterm birth rate in our study population was highest among Blacks at 8.7% (95% CI

6.6–10.7%), followed by 7.2% for Hispanic (95% CI 6.5–8.0%), 6.4% for White (95% CI 5.5–7.4%), and 5.6% for Asian newborns (95% CI 4.4–6.8%).

To evaluate the effect of birth weight, we selected 3430 infants born at GA of 39 weeks, which was the largest sample size with a specific GA (Table 1), and which removed the influence of GA on BW. Newborns in each race/ethnicity group were divided into three sub-groups (i.e., tertiles) based on BW. Within each race/ethnicity group, we compared MMA analytes between the highest and lowest BW tertile, with separate analyses for males and females to account for potential gender differences in BW. Results demonstrate higher C3 and C3/C2 among Hispanic and White infants in the highest BW tertile (Fig. 1E–F, Table 2). C3 and C3/C2 were also higher in Black females with higher BW, although these differences were not statistically significant in Black males. Asian infants with higher BW had higher C3/C2 but not C3. A similar trend of higher MMA marker levels in higher BW infants was also found for GA weeks 36 to 38 and 40.

4. Discussion

We recently identified a higher prevalence of Hispanic newborns among MMA false-positive cases (68.3%, 95% CI 64.3–72.4%) than among all newborns screened in California (52.2%) during the same time period [5], and hypothesized that this directly resulted from differences in levels of MMA markers between racial/ethnic groups. In support, we demonstrate significantly higher C3 and C3/C2 levels in Hispanic newborns compared to non-Hispanics, specifically compared to Black or White newborns. Interestingly, although C3 levels were generally higher in males (except for Blacks), the overall MMA falsepositive rate was not significantly higher for males (53.5% male, 95% CI 49.1–57.9%). This may reflect an inherently higher C3/C2 in females, likely due to lower C2.

Next, we explored the question of why Hispanic infants present with such elevated NBS markers. We hypothesized that newborn gestational age (GA) and birth weight (BW) could influence MMA marker levels (Table 2). Preterm birth, is known to influence NBS results [9], and indeed MMA false-positive cases were 4 times more likely to be born premature [5]. However, while preterm births are more common among Blacks than Hispanics, levels of C3 and C3/C2 were highest among Hispanics and lowest among Blacks, compared to the other groups. Therefore, the elevation of MMA screening markers seen in Hispanics is not entirely explained by differences in gestational age. The second covariate, low birth weight, has also been associated with falsepositive NBS results [9]. We previously reported an overall lower birth weight in MMA screen-positives, and birth weight was a top-ranked covariate for separating true- and false-positives using Random Forest [5]. Here we found a tendency for higher C3 and C3/C2 levels in healthy infants with higher birth weights. Thus, in healthy newborns, we found an inverse correlation for these two NBS covariates: decreased GA and increased BW were associated with increased MMA marker levels. Gestational age appears to be a stronger determinant for MMA marker levels as preterm birth was associated with lower birth weight (correlation coefficient between GA and logarithm of BW is 0.56, $P < .001$) and elevated markers, while full-term infants with higher birth weight had increased marker levels (Fig. 2). Finally, we compared the median BW of full-term MMA false-positives, which was found to be slightly higher (90 g) than that of healthy controls

(Table 1). However, this BW difference was not statistically significant. Further analyses in large multiethnic populations is needed to evaluate the effect of birth weight on NBS marker levels in different race/ethnicity groups.

In conclusion, here we provide evidence for a novel association between primary NBS metabolic disease markers and a race/ethnicity group. This finding could help explain why MMA false-positive results are more likely in Hispanic than in Black infants, which in turn raises the question of whether Black infants could be at a higher risk for falsenegative results. The four MMA patients with below-cutoff C3 and C3/2 in our study cohort were two White newborns (Cbl A or B, GA unknown, BW=1760 g; mut^{-}, GA=255 days, BW=2551 g) and two Hispanic newborns (mut^{-}, GA=273 days, BW=3500 g; unclassified, GA=244 days, BW=1558 g). Notably, two of the four patients had low BW (< 2500 g) which was found to be associated with decreased C3 and C3/2 and which could lead to false-negative results. However, the small number of Black newborns in this cohort (6.6%) limits further conclusions about potential false-negative cases and if different MMA marker thresholds should be considered. The causes of elevated MMA marker levels in Hispanic newborns are currently unknown. It is possible that systematic disparities based on socioeconomic status and race/ethnicity could affect GA, BW, and overall care, and that access to prenatal vitamins could affect maternal B12 levels [10,11]. It is also possible that genetic variants associated with race/ethnic groups and with variable biochemical and clinical phenotypes [12–14] contribute to the identified differences. These findings could inform screening and diagnostic procedures for MMA and potentially other inborn metabolic disorders.

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Abbreviations:

NBS	newborn screening
MMA	methylmalonic acidemia
MS/MS	tandem mass spectrometry

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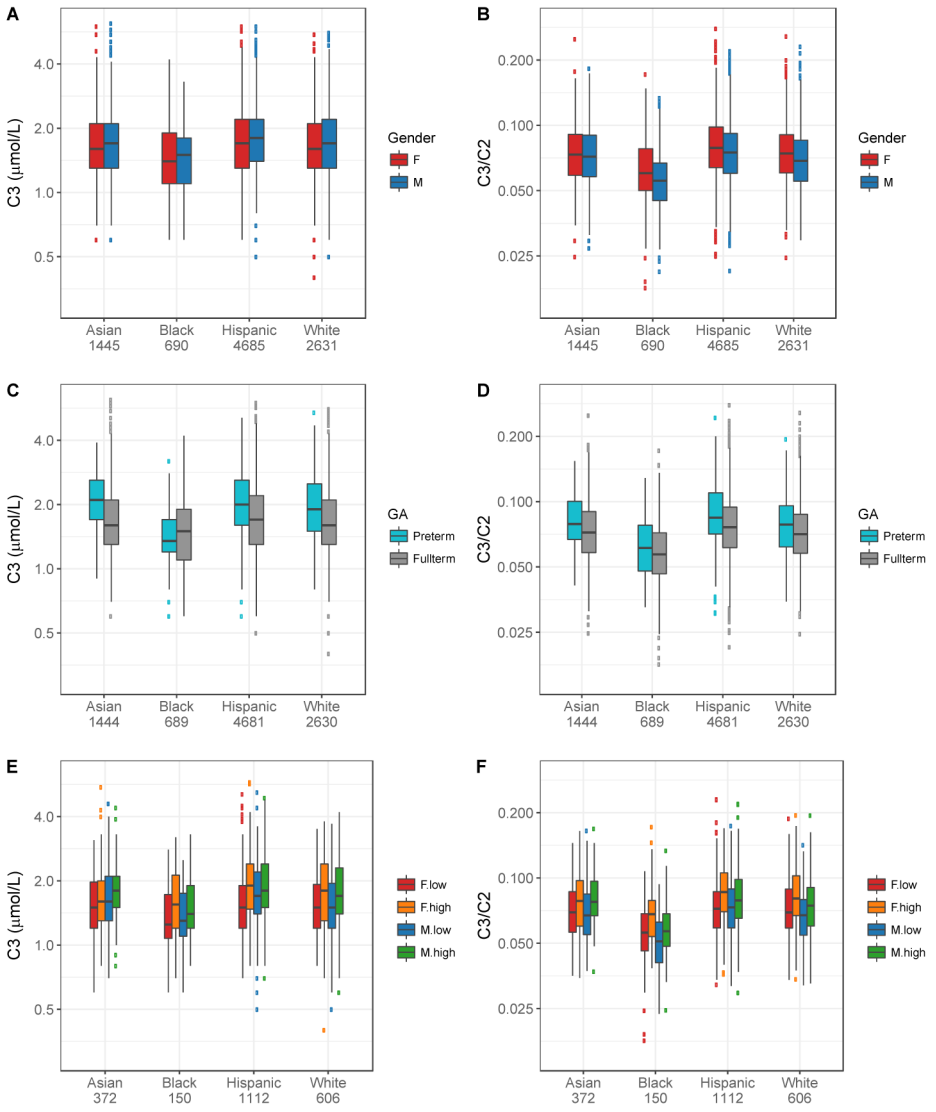

Fig. 1.

Methylmalonic acidemia (MMA) screening markers by race/ethnicity, gestational age (GA) and birth weight (BW). Differences in C3 (A) and C3/C2 (B) for four race/ethnicity groups, showing a tendency for higher values in Hispanic and lower values in Black infants. Within each race/ethnicity group, C3/C2 was relatively higher in females than males, while C3 was higher in males than females except in Black newborns. Comparison of GA within each group showed relatively higher C3 (C) and C3/C2 (D) in preterm than in term infants, except in Black newborns. Analysis of BW within each group (GA 39 weeks) showed higher C3 (E) and C3/C2 (F) in infants with a larger birth weight, except in Asian (C3) and Black male infants (C3 and C3/C2).

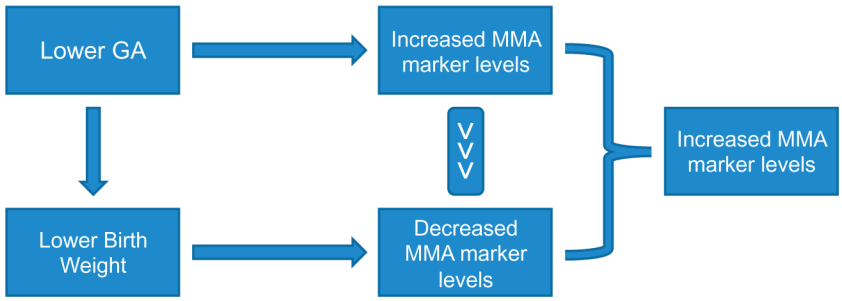

Fig. 2.

Association of MMA screening markers with gestational age and birth weight. Elevated MMA marker levels were found in preterm infants and in full-term infants with a higher birth weight. Preterm birth was associated with lower birth weight and increased MMA marker levels suggesting that gestational age is the stronger covariate compared to birth weight.

Table 1

Summary of NBS birth weight, gestational age and analytical data for screen-negative controls and MMA screen-positive cases.

	Controls			MMA false-positives			MMA true-positives		
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Preterm birth weight (grams)	2546	2558	590	2170	2135	753	2125	2045	578
Full-term birth weight (grams)	3379	3360	459	3428	3450	635	2989	2995	379
Gestational age (days)	273	273	12	259	266	27	270	274	19
C3 (μmol/L)	1.82	1.70	0.72	9.29	8.80	2.58	11.54	10.40	6.36
C3/C2	0.08	0.07	0.03	0.29	0.27	0.12	0.49	0.43	0.27
Gender[a]	4783 female/5194 male			232 female/267 male			49 female/54 male		

[a]Newborns without gender information were removed. MMA screen-positive cases were described previously [5].

Table 2

Comparison of C3 and C3/C2 levels between race/ethnic groups, and stratified by gender, gestational age and birth weight.

	Asian	Black	Hispanic	White
Difference in C3 levels between race/ethnic groups[a]				
Asian	–	0.39(0.30 to 0.49, *P* < .001)	− 0.13(−0.19 to 0.07, *P*=.19)	− 0.03(−0.10 to 0.03, *P*=.09)
Black	–	–	−0.52(−0.61 to −0.44, *P* < .001)	−0.43(−0.51 to −0.34, *P* < .001)
Hispanic	–	–	–	0.10(0.05 to 0.14, *P* < .001)
White	–	–	–	–
Difference of C3/C2 levels between race/ethnic groups[a]				
Asian	–	0.71(0.62 to 0.81, *P* < .001)	−0.16(−0.22 to −0.10, *P* < .001)	0.08(0.01 to 0.14, *P*=.20)
Black	–	–	−0.86(−0.94 to −0.78, *P* < .001)	−0.64(−0.73 to −0.55, *P* < .001)
Hispanic	–	–	–	0.23(0.19 to 0.28, *P* < .001)
White	–	–	–	–
Difference in analyte levels between male and female within each race/ethnic group[b]				
C3	0.15(0.04 to 0.25, *P*=.006)	−0.01(−0.16 to 0.14, *P*=.85)	0.08(0.03 to 0.14, *P*=.004)	0.09(0.01 to 0.16, *P*=.03)
C3/C2	−0.07(−0.17 to 0.04, *P*=.21)	−0.32(−0.47 to −0.17, *P* < .001)	−0.18(−0.24 to −0.13, *P* < .001)	−0.21(−0.29 to −0.14, *P* < .001)
Difference in analyte levels between preterm and term infants within each race/ethnic group[c]				
C3	0.64(0.40 to 0.88, *P* < .001)	−0.11(−0.39 to 0.17, *P*=.43)	0.37(0.25 to 0.49, *P* < .001)	0.40(0.23 to 0.57, *P* < .001)
C3/C2	0.34(0.11 to 0.58, *P* < .001)	0.14(−0.14 to 0.42, *P*=.28)	0.44(0.32 to 0.56, *P* < .001)	0.35(0.18 to 0.51, *P*=.002)
Difference in analyte levels between infants with higher and lower BW born at GA of 39 weeks[d]				
C3 (f)	0.25(−0.05 to 0.56, *P*=.096)	0.53(0.06 to 1.01, *P*=.026)	0.56(0.39 to 0.73, *P* < .001)	0.36(0.13 to 0.59, *P*=.002)
C3 (m)	0.26(−0.02 to 0.54, *P*=.066)	0.40(−0.06 to 0.85, *P*=.082)	0.22(0.06 to 0.39, *P*=.008)	0.42(0.19 to 0.65, *P* < .001)
C3/C2 (f)	0.41(0.11 to 0.71, *P*=.008)	0.75(0.26 to 1.23, *P*=.002)	0.53(0.36 to 0.70, *P* < .001)	0.47(0.24 to 0.71, *P* < .001)
C3/C2 (m)	0.55(0.26 to 0.83, *P* < .001)	0.39(−0.06 to 0.85, *P*=.087)	0.28(0.11 to 0.44, *P*=.001)	0.38(0.16 to 0.61, *P* < .001)

Pairwise comparison of groups was performed using Cohen's d with the 95% CI and *P* value of each *t*-test in parenthesis.

[a] Positive Cohen's d indicates higher C3 or C3/C2 values in row group than in column group.

[b] Positive Cohen's d indicates higher C3 or C3/C2 values in males than in females.

[c] Positive Cohen's d indicates higher C3 or C3/C2 values in preterm than in full-term infants.

[d] Positive Cohen's d indicates higher C3 or C3/C2 values in newborns in the highest BW tertile than newborns in the lowest BW tertile (Abbreviations: f=female, m=male).