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ORIGINAL ARTICLE

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A nationwide retrospective observational study of population newborn screening for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in the Netherlands

Emmalie A. Jager[1] | Myrthe M. Kuijpers[1] | Annet M. Bosch[2] | Margot F. Mulder[3] | Estela R. Gozalbo[4] | Gepke Visser[5] | Maaike de Vries[6] | Monique Williams[7] | Hans R. Waterham[2] | Francjan J. van Spronsen[1] | Peter C. J. I. Schielen[8] | Terry G. J. Derks[1]

[1]Section of Metabolic Diseases, Beatrix Children's Hospital, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

[2]Pediatric Metabolic Diseases, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Pediatrics, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[4]Department of Pediatrics and Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands

[5]Department of Metabolic Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

[6]Institute for Genetic and Metabolic Disease, Department of Pediatrics, Radboud University Medical Centre Nijmegen, Nijmegen, The Netherlands

[7]Center for Lysosomal and Metabolic Diseases, Department of Pediatrics, Erasmus Medical Centre, Rotterdam, The Netherlands

[8]Reference laboratory Neonatal Screening, Centre for Public Health Research, National Institute of Public Health and Environment (RIVM), Bilthoven, The Netherlands

Correspondence
Terry G. J. Derks, Section of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, PO Box 30 001, 9700 RB Groningen, The Netherlands.
Email: t.g.j.derks@umcg.nl

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Abstract

To evaluate the Dutch newborn screening (NBS) for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency since 2007, a nationwide retrospective, observational study was performed of clinical, laboratory and epidemiological parameters of patients with MCAD deficiency born between 2007 and 2015. Severe MCAD deficiency was defined by *ACADM* genotypes associated with clinical ascertainment, or variant *ACADM* genotypes with a residual MCAD enzyme activity <10%. Mild MCAD deficiency was defined by variant *ACADM* genotypes with a residual MCAD enzyme activity ≥10%. The prevalence of MCAD deficiency was 1/8300 (95% CI: 1/7300-1/9600). Sensitivity of the Dutch NBS was 99% and specificity ~100%, with a positive predictive value of 86%. Thirteen newborns with MCAD deficiency suffered from neonatal symptoms, three of them died. Of the 189 identified neonates, 24% had mild MCAD deficiency. The acylcarnitine ratio octanoylcarnitine (C8)/decanoylcarnitine (C10) was superior to C8 in discriminating between mild and severe cases and more stable in the first days of life. NBS for MCAD deficiency has a high sensitivity, specificity, and positive predictive value. In the absence of a golden standard to confirm the diagnosis, the combination of acylcarnitine (ratios), molecular and enzymatic studies allows risk stratification. To improve evaluation of NBS protocols and clinical guidelines, additional use of acylcarnitine ratios and multivariate pattern-recognition software may be reappraised in the Dutch situation. Prospective recording of NBS and follow-up data is warranted covering the entire health care chain of preventive and curative medicine.

KEYWORDS

acylcarnitine, inborn errors of metabolism, medium-chain acyl-CoA dehydrogenase deficiency, neonatal screening, prevalence

1 | INTRODUCTION

Medium-chain acyl-CoA dehydrogenase (MCAD, EC 1.3.8.7) facilitates the first step in the mitochondrial β-oxidation of CoA-esters of medium-chain fatty acids.[1] MCAD deficiency (#OMIM 201450) is the most common inherited defect of mitochondrial fatty acid oxidation and is potentially fatal. Acute symptoms and signs like encephalopathy and coma usually occur in infancy, biochemically associated with hypoketotic hypoglycaemia.[2] Outcomes are excellent after early establishment of the diagnosis and consequent management, which in the Netherlands includes the advice on the avoidance of prolonged fasting[3-6] and an emergency regimen during intercurrent illness.

Testing for MCAD deficiency has been implemented in population newborn bloodspot screening (NBS) programs in many countries from the late 1990s.[7] Concentrations of (medium-chain length) acylcarnitines and their molar ratios have been used as NBS parameters for MCAD deficiency. Among countries, NBS protocols vary with respect to the day of blood collection, analytical aspects, screening parameters, cut-off values, the use of post-analytical diagnostic algorithms based on multivariate pattern recognition and follow-up protocols to establish the definitive diagnosis.[7-9] In the Netherlands, MCAD deficiency was introduced in the national NBS program in 2007, after nationwide studies on the natural history,[10] the epidemiology,[11] a prospective pilot NBS study in the northern part of the country[12] and an economic evaluation.[13]

The fundamental purpose of population NBS (for MCAD deficiency) is to rapidly diagnose newborns in order to prevent or reduce irreversible morbidity and mortality by early treatment. It was demonstrated that our national NBS program for MCAD deficiency identifies more patients,[11,14] with previously unreported *ACADM* genotypes, than historically recognized through clinical ascertainment.[14,15] Case definition of MCAD deficiency has, therefore, become more difficult.[16-18] Also, it is recognized that some MCAD deficient patients display acute (fatal) symptoms, before NBS results have become available, or even before blood samples have been taken.[16,19-22]

To evaluate the Dutch population NBS for MCAD deficiency since 2007, we performed a nationwide retrospective, observational study of clinical, laboratory and epidemiological parameters.

2 | METHODS

2.1 | Ethics approval

For this retrospective cohort study, the Medical Ethical Committee (METc) of the University Medical Center Groningen provided a waiver indicating that the Medical Research Involving Human Subjects Act was not applicable and official study approval by the METc was not required (METc 2015/540).

This article does not contain any studies with animal subjects performed by the any of the authors.

2.2 | Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, last revision in 2013 (5).

For the Dutch NBS, *written* parental informed consent is not required, as described in detail elsewhere.

The retrospective study is exempted from formal IRB/Ethics board review, because the Medical Research Involving Human Subjects Act is not applicable. Under these circumstances and when data are used anonymously, individual subject's informed consent is not required, as per institutional and national rules.

2.3 | Dutch population NBS protocol for MCAD deficiency and follow-up

The Dutch NBS is coordinated by the Centre for Population Screening of the National Institute for Public Health and the Environment (RIVM). Filter paper dried blood spots (DBS) are collected between 72 and 168 hours after birth (managed in 98.8% of the newborns) and sent to one of the five national screening laboratories for analysis. The national laboratories perform quality monitoring quarterly. In the Netherlands participation in the population NBS program is ~99.5%.[23]

For MCAD deficiency, the primary screening marker, on which referral is solely based, is the DBS concentration of octanoylcarnitine (C8 cut off ≥0.5 μmol/L), derived from a conservative estimate based on literature[24,25] and adapted after a prospective pilot screening in the northern part of the Netherlands.[10] The ratio between C8 and decanoylcarnitine (C8/C10) (cut off >5.0) is reported as a secondary marker, whereas C2, C6, and C10:1 are reported as tertiary markers. However, these markers do not influence the decision-taking progress (www.rivm.nl). The Dutch NBS algorithm for MCAD deficiency has not changed during this study.

Approximately 98% of the newborns with abnormal screening results are reported within 1 week after birth to the general practitioner (GP) and the nearest metabolic center. After the immediate referral by the GP, the newborns are seen within 24 hours for clinical and laboratory follow-up in the metabolic center. Here diagnosis is established according

to the Dutch standard of care protocol for MCAD deficiency[26] by a combination of:

- Increased medium-chain (C6 to C12) plasma acylcarnitines, especially C8, and relevant ratio's (such as C8/C10 and C8/C2).
- Increased urinary organic acid excretions, particularly hydroxy acids, dicarboxylic acids and glycine derivatives of hexanoic-, suberic-, and phenylpropionic acid.
- MCAD enzyme activity, determined in leucocytes or lymphocytes isolated from freshly collected blood, with an HPLC-based assay using 3-phenylpropionyl-CoA as a substrate, as published previously.[27-29] Assays are performed in duplo (interassay variation <10%). Residual enzyme activities are expressed as percentage of healthy controls.
- *ACADM* gene mutation-analysis.

In the Netherlands, the costs of the above-mentioned confirmatory follow-up testing are covered by health insurance companies.

2.4 | Cohort

Included were all Dutch children with an established diagnosis of MCAD deficiency born in 2007 up to and including 2015. Data were retrieved from the RIVM, the Dutch metabolic centers, and their collaborating laboratories. The Dutch Diagnosis Registration Metabolic Diseases Database (www.ddrmd.nl), a national registry for positive NBS screening results of patients with confirmed diagnosis of an IEM, and the Dutch Pediatric Surveillance Unit[30] served a control for possible false-negatives. Acylcarnitine concentrations from 1000 healthy newborns were retrieved as a reference cohort from the RIVM.

2.5 | Data analysis

Based on previously published methods[14] severe MCAD deficiency was defined by *ACADM* genotypes associated with clinical ascertainment, or *ACADM* genotypes *not* associated with clinical ascertainment (variant *ACADM* genotypes) with a residual MCAD enzyme activity <10%. Mild MCAD deficiency was defined by variant *ACADM* genotypes with a residual MCAD enzyme activity ≥10%.

Prevalence (P) was calculated by dividing the number of patients with MCAD deficiency diagnosed between 2007 and 2015 by the total number of live births (N). The total number of live births was acquired from the Dutch Central Bureau for Statistics (ww.cbs.nl). The confidence interval (CI) was calculated as:

$$95\%CI = \mathrm{P} \pm 1.96 \times ((\mathrm{P} \times (1-\mathrm{P})/N))^{1/2}$$

The *z*-scores of individual NBS screenings parameters (μ) were established with the mean (X) and SD (σ) derived from the reference population using:

$$Z = \frac{\mathrm{X} - \mu}{\sigma}$$

Data analysis and visualization was performed using IBM SPSS Statistics for Windows, (IBM Corp. Released 2016. Version 24.0. Armonk, New York), GraphPad Prism software (GraphPad Software Inc. Released 2016. Version 7.02. San Diego, California) and R (Version 3.4.0, Vienna, Austria). The not normally distributed data was described with medians and interquartile ranges (IQR) and analyzed using Mann-Whitney *U* or Kruskal-Wallis test, followed by Dunn's multiple comparison test. The sensitivity and specificity of the screening parameters were calculated and illustrated by Receiver Operating Characteristic (ROC) curves. The areas under the ROC curves (AUCs of ROCs) were analyzed using Delong test and the 95% confidence interval was generated (CI 95%). Optimal cut-offs were established using R package "OptimalCutpoints" version 1.1-3.[31] Minimum distance to the top left corner was calculated using:

$$d = \sqrt{(1-\text{sensitivity})^2 + (1-\text{specificity})^2}$$

The Youden index was established using:

$$J = (\text{sensitivity} + \text{specificity}) - 1$$

Differences were considered significant when $P < .05$.

3 | RESULTS

3.1 | Epidemiology

A total of 1 614 278 DBS were analyzed, 219 subjects were referred after positive screening results and 189 eventually diagnosed with MCAD deficiency. Two false-negatives cases were identified, and three patients were diagnosed post-mortally. The sensitivity of the Dutch NBS program for MCAD deficiency was 99% and the specificity ~100%, with a positive predictive value (PPV) of 86%. With 1 608 333 live births, the prevalence of MCAD deficiency was 1/8300 (95% CI: 1/7300-1/9600) (Table 1).

Three c.985A > G *ACADM* homozygotes died on the second ($n = 1$) and third ($n = 2$) day of life, before DBS collection. Hence, these children could not be identified by the NBS program. Additionally, eight patients identified by the

TABLE 1 Epidemiology of MCAD deficiency in the Netherlands

MCAD deficiency	Frequencies (95% CI)
ACADM mutation carrier frequency[(a)]	1/55 (1/46-1/68)
Expected prevalence[(a)]	1/12100 (1/8450-1/18500)
Observed prevalence 1985-1999[(b)]	1/27400 (1/23000-1/33900)
Observed prevalence NBS 2007-2010[(c)]	1 8750 (1/7210-1/11130)
Live births 2007-2015[(d)]	1 608 333
Expected MCAD deficient patients 2007-2015[(a)]	183 (144-223)
Positive screenings NBS 2007-2015	219
MCAD deficiency patients 2007-2015	194
Observed Prevalence 2007-2015	1/8300 (1/7300-1/9600)

Legend: (a) according to Reference 32 (b) according to Reference 11 (c) according to Reference 14 (d) CBS, 2016.

NBS, already suffered from symptoms during the neonatal period. *ACADM* molecular studies revealed c.985A>G homozygosity ($n = 5$) and compound heterozygosity for the c.789A>G mutation (with c.985A>G [$n = 1$] and c.233 T>C [$n = 1$]) in these cases. From one patient with neonatal symptoms, in whom *ACADM* gene mutation-analysis data is lacking, residual enzyme activity was 0%. Five of these patients with neonatal symptoms had been admitted to the hospital before NBS test results became available, three with documented hypoglycaemia.

In eight newborns frequent feeds were advised from birth because of a family history of MCAD deficiency. Interestingly, two of these patients, both compound heterozygotes for the variant c.985A>G and c.199T>C *ACADM* mutations, were not detected by the NBS program (sampling days not documented). Their C8 concentrations were 0.22 μmol/L and 0.35 μmol/L (respectively 10 and 17 *z*-scores above the median). The C8/C10 ratios were 2.4 and 2.3 (respectively 3.4 and 3.6 *z*-scores above the median), whereas the C8/C2 ratio was 0.3 in one of the cases (23 *z*-scores above the median). In the second case, the C8/C2 ratio was missing. The residual enzyme activities were 42% and 48%, respectively. Therefore, these two false-negatives were categorized as mild MCAD deficiency.

3.2 | *ACADM* genotypes and residual enzyme activity

Data on *ACADM* gene mutations analysis were available for 88% (167/191) of the screened patients. The c.985A>G *ACADM* allele frequency was 75% (252/334); 58% (97/167) of the patients were homozygous for this classic mutation, 35% (58/167) were compound heterozygotes. Five previously unreported *ACADM* mutations were identified; c.1229T>C, c.614C>T, c.653C>G, c.745G>A, appearing in combination with c.985A>G, and c.794_803delinsTTTAAA combined with c.600-18G>A. None of these patients were documented to have neonatal symptoms.

Combined data on both residual MCAD enzyme activities and *ACADM* genotypes could be retrieved from 83% (159/191) of the screened patients (Table SS1). In total, 24% (38/159) were categorized as mild MCAD deficiency, of whom 61% (23/38) had residual MCAD enzyme activities ≥30%. Nine of the patients with variant *ACADM* genotypes were categorized as severe MCAD deficiency, as they displayed residual MCAD enzyme activities <10%. Three individuals carrying the c.799G>A mutation[33,34] displayed residual MCAD enzyme activities around 10% (Table S1).

3.3 | Population NBS screening parameters

Table 2 presents the population NBS acylcarnitine levels for the Dutch MCAD deficiency cohort 2007-2015. The median C8 concentration, C8/C10 and C8/C2 ratio all statistically differed (Mann-Whitney U, $P < .05$) between true-positives and the control cohort (Table 2).

Comparing the AUC of ROC (CI 95%) of C8 (0.94 [0.90-0.98]), C8/C10 [0 .79 (0.67-0.91]) and C8/C2 (0.92 [0.87-0.96]) for MCAD deficiency patients and NBS false-positives, showed that C8 and C8/C2 were more accurate at predicting MCAD deficiency than C8/C10 (Delong, $P < .05$) (Figure 1A). Interestingly, comparing the AUC of

TABLE 2 Population NBS parameters for MCAD deficiency

Group (*n*)	C8*	C8/C10	C8/C2*
TP	2.74	12.3	0.20
($n = 189$)	(1.57-4.75)	(9.0-13.9)	(0.09-0.31)
Severe	3.38	13.1	0.24
($n = 121$)	(1.9-5.4)	(11.6-14.3)	(0.16-0.34)
Mild	1.12	3.10	0.05
($n = 38$)	(0.72-1.81)	(2.7-3.7)	(0.03-0.08)
FP	0.61	2.3	0.03
($n = 30$)	(0.55-0.8)	(0.7-7.7)	(0.03-0.04)
FN	0.29	2.35	0.03
($n = 2$)	(0.22-0.35)	(2.3-2.4)	0.03
Control	0.04	0.8	0.002
($n = 1000$)	(0.03-0.05)	(0.7-1.0)	(0.002-0.003)

Legend: median values (IQR) displayed for population NBS parameters for MCAD deficiency in the Dutch NBS cohort 2007-2015. TP, true-positives; FP, false-positives. *no significant difference was found between the value of TP and FP (Kruskal-Wallis, $P > .05$). The severe and mild group consist of the patients from which the combined data on both residual MCAD enzyme activities and *ACADM* genotypes were available ($n = 159$).

ROC (CI 95%) of C8, C8/C10 and C8/C2 between severe and mild MCAD deficient patients, that is, 0.87 (0.80-0.93), 0.98 (0.94-1.00) and 0.94 (0.90-0.99), respectively, both ratios were found to predicted disease severity more accurately compared to C8 (Delong, $P < .05$) (Figure 1B).

Application of C8, C8/C10 and C8/C2 as primary screening markers to solely identify severe MCAD deficiency (cut-off values maximizing sensitivity established at; C8: 0.77 μmol/L [100% sensitivity and 97% specificity]; C8/C10: 3.2 [100% sensitivity and 97 specificity] and C8/C2: 0.038 [100% sensitivity and 97% specificity]) would have resulted in the elimination of 80% of the false positives and 58% (22/39) of the mild MCAD deficiency cases. These mild MCAD deficiency subjects would have represented residual MCAD enzyme activities ranging between 11% and 65% and most would have carried the *ACADM* c.985A>G/c.199T>C genotype ($n = 10$).

The median age at NBS blood sampling of the MCAD deficiency patients ($n = 183$; missing data in 6 cases) was 4 days (range: 3-6). Median (IQR) concentrations of C8 are highest when NBS was performed earlier; 5.9 (3.1-9.2), 3.2 (1.8-4.8), 1.8 (1.3-3.3) and 1.5 (1.1-2.4) μmol/L on day three, four, five and six, respectively (Kruskal Wallis, $P < .05$) (Figure 2A). Median (IQR) C8/C2-ratios also declined from 0.30 (0.21-0.39) on day three to 0.16 (0.11-0.21) on day six, albeit not significantly (Kruskal Wallis, $P > .05$) (Figure 2B). The median (IQR) C8/C10-ratio ranged between 12.55 (11.23-15.00, on day three) to 12.35 (10.73-12.88, on day 5) (Kruskal Wallis, $P > .05$) (Figure 2C).

4 | DISCUSSION

The present study is a nationwide retrospective, observational study of clinical, laboratory and epidemiological parameters of population NBS for MCAD deficiency in the Netherlands with a high acceptance rate. During nearly a decade in which 1.6 million DBS were studied, we identified 194 patients of whom most were characterized at both an enzymatic and molecular level.

The sensitivity of the Dutch NBS program was 99% and the specificity ~100%, with a PPV of 86%. This high-performance rate of the Dutch NBS is partly explained by the Dutch follow-up protocol to confirm the diagnosis. The prevalence of MCAD deficiency is 1/8300 (CI 95%: 1/7300-1/9600), in agreement with the estimate based on the *ACADM* c.985A>G carrier frequency in the general population and the assumption of a 94% allele frequency for this common mutation in clinically ascertained cases.[14,32] Although the Dutch NBS program is thus very suitable to identify MCAD deficiency, it can be discussed whether the current follow-up approach only identifies those neonates prone to morbidity or mortality due to the disorder.

To date, there is no gold standard to confirm the diagnosis of MCAD deficiency. The Dutch follow-up protocol allows retrospective risk assessment based on clinical and

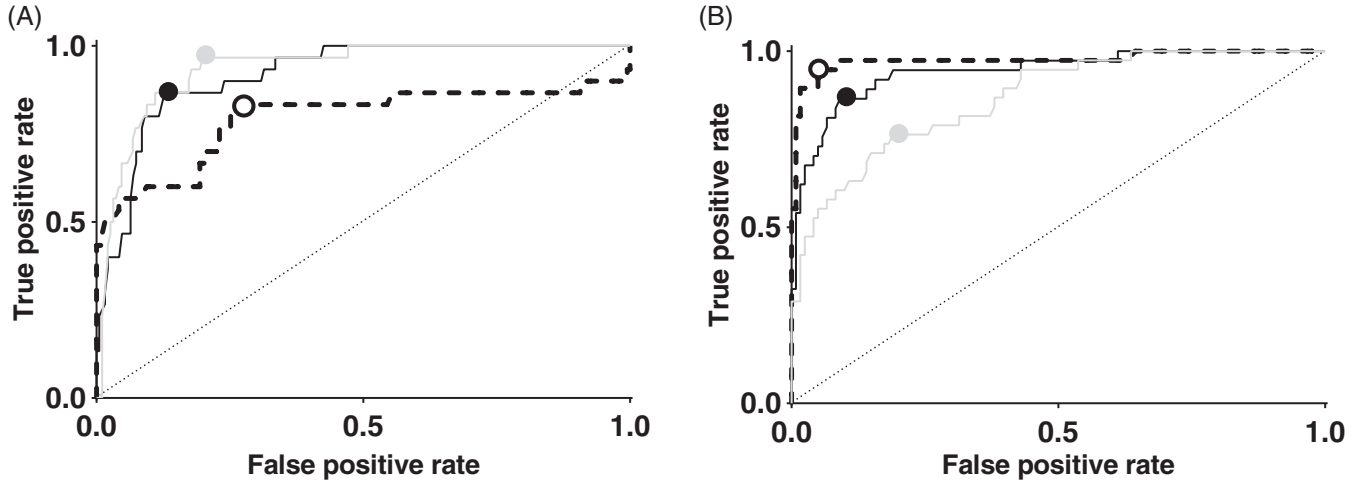

FIGURE 1 ROC curves of the population NBS markers for differentiating (A) MCAD deficiency patients from false-positives and (B) between severe and mild MCAD deficiency. Legend: The true positive rate (sensitivity) and false positive rate of the C8 concentration (μmol/L), C8/C10 ratio and C8/C2 ratio between (A) screened MCAD deficiency patients (TP + FN, $n = 1191$) and false-positives ($n = 30$). The gray line represents C8, the black dashed line represents C8/C10, and the black line represents C8/C2. the points represent the optimal thresholds. Optimal thresholds were established at C8 = 1.35 μmol/L (sensitivity: 97%, specificity: 80%, $d = 0.20$ $J = 0.77$), C8/C10 = 9.8 (sensitivity 83%, specificity: 73%, $d = 0.32$, $J = 0.56$) and C8/C2 = 0.094 (sensitivity: 87%, specificity: 88%, $d = 0.18$, $J = 0.75$). (B) between severe MCAD deficiency ($n = 121$) and mild MCAD deficiency ($n = 38$) patients. Optimal thresholds were established at C8 = 1.76 μmol/L (sensitivity: 76%, specificity: 80%, $d = 0.31$, $J = 0.56$), C8/C10 = 8.4 (sensitivity: 95%, specificity: 95%, $d = 0.071$, $J = 0.90$) and C8/C2 = 0.095 (sensitivity: 86%, specificity: 90%, $d = 0.17$, $J = 0.76$)

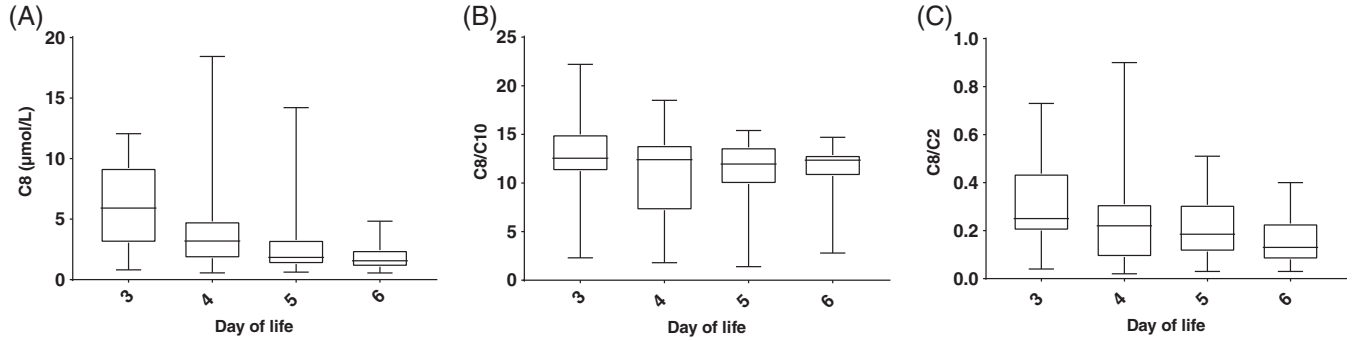

FIGURE 2 NBS screening parameters according to the day at which the NBS bloodspot was obtained in screened MCAD deficiency patients for (A) C8, (B) C8/C10 and (C) C8/C2. Legend: the values of the various screening markers, (A) C8 concentration (µmol/L), (B) C8/C10 ratio (C) C8/C2 ratio, according to the of life day at which the NBS bloodspot was obtained (day 3 $n = 18$, day 4 $n = 111$, day 5 $n = 36$, day 6 $n = 20$, missing data in 6 cases). Whiskers show the minimal and maximum values

multiple laboratory parameters. *ACADM* gene sequencing and identification of bi-allelic mutations is frequently used for confirmatory testing after initial abnormal NBS results for MCAD deficiency. However, already before the introduction of NBS programs, MCAD deficiency had a poor genotype-phenotype correlation.[10,15,35,36] Some *ACADM* genotypes (such as c.985A > G homozygosity) can cause a clinical phenotype. For positively screened newborns with variant *ACADM* genotypes, both C8/C10 and in vitro studied residual MCAD enzyme activity can be of additional value for risk prediction.[14,37] The c.985A > G *ACADM* homozygotes have residual activities <1% and *ACADM* genotypes resulting in residual activities <10% are observed in patients with neonatal clinical presentations, hypoglycaemias and more frequent preventive hospital admissions.[14] Furthermore, heterozygous carriers of the c.985A>G mutation have residual enzyme activities >22%.[37-39]

In this study, we found that nearly a quarter of the neonates identified by the Dutch NBS program have mild MCAD deficiency. If mild MCAD deficiency cases would be excluded, the prevalence would still be in line with the estimate based on the c.985A>G *ACADM* carrier frequency in our country. It can be questioned whether mild MCAD deficiency cases, such as compound heterozygotes for the c.985A>G and c.199T>C *ACADM* mutations, realistically carry *clinical* risks or only demonstrate a *biochemical* variation causing unnecessary anxiety, medical interventions, and follow-up.[48,49] Long-term clinical follow-up studies are warranted to assess the clinical consequences of mild MCAD deficiency.

This study demonstrates that -for those countries where enzyme activity is not measured routinely- the C8/C10 ratio is a powerful marker for early discrimination between severe and mild MCAD deficiency cases (Figure 1B). Multivariate pattern-recognition software (including acylcarnitine ratios), such as from the Collaborative Laboratory Integrated Reports (CLIR), could be used alternatively to differentiate between severe and mild MCAD deficiency cases[40] and circumvent the definition of an exact-cut off.[33]

This study demonstrated that C8/C10 is more stable in the first days of life than C8 in subjects with MCAD deficiency. It has been demonstrated that C8 concentrations do not vary between 4 and 6 days of life in normal, healthy neonates.[41] Earlier studies reported that the C8/C10 ratio was neither influenced by metabolic stress nor by nutritional state,[42,43] in contrast to the C8 concentration.[8,44,45] In various NBS protocols across the world C8/C10 is implemented as a second parameter.[8]

Despite the high clinical awareness in our country and early detection by NBS, this study identified at least three patients who died before DBS could be obtained. We recently reported that the C8 concentration and C8/C10 ratio in cord-blood from MCAD-deficient patients are already abnormal and the C8/C10 ratio is already remarkably stable during the first 2 days of life.[21,22] This indicates that earlier screening using C8/C10 ratio may enable rapid diagnosis of MCAD deficiency. However, changing the timing for one disorder would affect (the timing of) the entire health care chain of a national NBS program, including cut-off values for the remaining disorders.

Some methodological issues of this study should be addressed. Several factors are reported to influence acylcarnitine profiles, such as (very) low birthweight and prematurity.[25,45-47] These factors were not systematically collected in this study, but deserve future attention using multivariate pattern-recognition software, including age in hours at sampling. Second, laboratory follow-up has differed among the patients. The enzyme analysis is performed in leukocytes or lymphocytes, even though the latter seems preferable.[14,37] Recently a national central facility for integrated and continuous documentation of the population NBS program was implemented (NEORAH). If follow-up data would be incorporated, this holds the potential of future (international) data sharing, covering the entire health care chain of preventive and curative medicine.

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Author contributors

E.J. conceptualized and designed the retrospective cohort study, coordinated and performed data collection, performed data analysis and interpretation, drafted and revised the manuscript. M.K. conceptualized and designed the retrospective cohort study, coordinated and performed data collection, contributed to data analysis and critically reviewed the manuscript. A.B., M.M., E.G., G.V., M.V., M.W., H.W., and F.S. contributed to data collection and critically reviewed the manuscript. P.S. designed the retrospective cohort study, coordinated data collection, supervised data analysis and interpretation and critically reviewed the manuscript. T.D. conceptualized and designed the retrospective cohort study, coordinated data collection, supervised data analysis and interpretation, drafted and revised the manuscript. All authors approved the final manuscript submitted.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Emmalie A. Jager https://orcid.org/0000-0002-7360-8215
Terry G. J. Derks https://orcid.org/0000-0002-7259-1095

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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