JIMD Reports
DOI 10.1007/8904_2016_30

RESEARCH REPORT

Primary Carnitine Deficiency: Is Foetal Development Affected and Can Newborn Screening Be Improved?

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Received: 21 September 2016 / Revised: 15 November 2016 / Accepted: 23 November 2016 / Published online: 20 January 2017

Abstract Primary carnitine deficiency (PCD) causes low levels of carnitine in patients potentially leading to metabolic and cardiac symptoms. Newborn screening for PCD is now routine in many countries by measuring carnitine levels in infants. In this study we report Apgar scores, length and weight in newborns with PCD and newborns born to mothers with PCD compared to controls. Furthermore we report how effective different screening algorithms have been to detect newborns with PCD in the Faroe Islands.

Results: Newborns with PCD and newborns born to mothers with PCD did not differ with regard to Apgar scores, length and weight compared to controls. Newborns with PCD and newborns born to mothers with PCD had significantly lower levels of free carnitine (fC0) than controls. Screening algorithms focusing only on fC0 had a high rate of detection of newborns with PCD. Sample collection 4–9 days after birth seems to result in a higher detection rate than the current 2–3 days.

Conclusion: The clinical status at birth in infants with PCD and infants born to mothers with PCD does not differ compared to control infants. Screening algorithms for PCD should focus on fC0, and blood samples should be taken when the maternal influence on fC0 has diminished.

Communicated by: John H Walter, MD FRCPCH

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Introduction

The Faroe Islands are a small group of islands in the North Atlantic with a population of only 50,000 inhabitants of British and Norse descent (Jorgensen et al. 2002, 2004; Als et al. 2006). Primary carnitine deficiency (PCD, OMIM #212140) is a prevalent inherited disorder in the Faroe Islands with a prevalence of 1:300 compared to an estimated prevalence of 1:20,000–120,000 around the world (Koizumi et al. 1999; Wilcken et al. 2003; Magoulas and El-Hattab 2012; Rasmussen et al. 2014a, b). PCD (also known as, e.g., CTD and CUD) is an autosomal recessive disorder of fatty acid oxidation caused by a dysfunctional carnitine transporter (OCTN2) coded by the *SLC22A5* gene on chromosome 5 (Nezu et al. 1999). Patients continually lose carnitine in urine because of impaired renal reabsorption of filtered carnitine, which causes low levels of carnitine in patients (Scaglia et al. 1998; Longo et al. 2006; Rasmussen et al. 2015). Symptoms range from no symptoms to fatigue, cardiomyopathy and even fatal cardiac arrhythmia. A majority of the cases reported in the literature of symptomatic PCD patients have been among children (Tein 2003; Stanley 2004; Longo et al. 2006; Magoulas and El-Hattab 2012; Rasmussen et al. 2013). However, it is not known if newborns with PCD or newborns born to mothers with PCD are affected by abnormally low carnitine levels during foetal development

and at birth. Neonatal screening for PCD was routinely implemented in the Faroese newborn screening programme in 2009, but was preceded by a pilot screening study from 2002 to 2009 (Lund et al. 2012). The fear that there were undiagnosed PCD patients in the population led to the implementation of a nationwide screening programme in 2009 to uncover as many patients as possible (Rasmussen et al. 2014a). The screening programme had two parts, one being an offer to all inhabitants to have their blood carnitine levels determined and another being a retrospective newborn screening programme analysing all dried blood spot (DBS) samples from the newborn screening 1986–2001, which were available from the Danish Neonatal Screening Biobank (Rasmussen et al. 2014a). The extensive and ongoing screening programmes have led to the diagnosis of approximately 160 patients with PCD in the Faroe Islands, including children and adults.

In this study we investigate how newborns with PCD and newborns born to mothers with PCD compare to controls with regard to birth weight, length and Apgar scores. Furthermore we show how effective the newborn screening programmes have been detecting newborns with PCD and what role differences in screening algorithms and cut-off values have played.

Materials and Methods

Patients verified by genetic analysis to have PCD and children of mothers with PCD were included, as well as controls with normal free carnitine (fC0) levels matched by gender – all born between 1979 and 2011. Data on birth weight, length, Apgar scores, parity and gestational age were collected from birth charts filled out by midwives from 1979 to 2011 (Apgar 1953; Finster and Wood 2005).

fC0 levels from the included subjects measured unbutylated by tandem mass spectrometry in the population-based voluntary screening programme from 2009 and onwards were collected when available (Rasmussen et al. 2014a).

Prospective Newborn Screening

Carnitine levels in DBS samples from the prospective newborn screening were either analysed as described previously as part of a pilot project of extended newborn screening in babies from 2002 to 2008 or as part of the routine newborn screening from 2009 and onwards (Lund et al. 2012). Analyses were done by tandem mass spectrometry using an fC0 cut-off, which from 2002 to 2008 was adjusted when necessary according to medians and percentiles of measured carnitine levels and varied from 5.7 to 6.3 μmol/L, while being 5 μmol/L from 2009. DBS samples for newborn screening were obtained 4–9 days after birth and analysed butylated until 2009 when the recommended time to obtain a sample was changed to 2–3 days after birth and the analysis was done unbutylated, which remains unchanged until today (Lund et al. 2012).

During most of the pilot period 2002–2008 a lower cut-off value for acetyl-carnitine (C2) was also used alongside fC0 when evaluating the samples for PCD.

Retrospective Screening

DBS samples from Faroese newborns collected for neonatal screening are stored at minus 24°C in the Danish Newborn Screening Biobank (Norgaard-Pedersen and Simonsen 1999; Norgaard-Pedersen and Hougaard 2007). All DBS samples from children born in the Faroe Islands dating back to 1986 and onwards, which had not previously been analysed for carnitine levels, were also retrospectively analysed unbutylated in order to determine carnitine levels and reveal subjects born with abnormally low levels of carnitine. The stored DBS samples were analysed using tandem mass spectrometry as previously described, but the lower cut-off value for fC0 was set higher at 9 μmol/L to adjust for possible hydrolysis of acylcarnitines to free carnitine known to occur on storage of DBS (Lund et al. 2012; Strnadova et al. 2007).

Statistics

Data analysis was performed using IBM® SPSS® Statistics Version 19 (SPSS Inc., Chicago, IL, USA). All continuous variables were expressed as mean (standard deviation). One-way Anova was used to test for a significant difference in mean between the three groups – Tukey HSD test was used to test the difference between individual groups. Level of significance was set at $p < 0.05$.

Results

The study included 79 PCD patients, 56 subjects born to untreated mothers with PCD and 313 controls (Table 1). A total of 212 females and 236 males were included.

Length, Weight, Gestational Age and Parity

There was no significant difference in length ($p = 0.98$) and weight ($p = 0.38$) between the groups of newborns with PCD, newborns born to mothers with PCD and controls (Table 1). The groups neither differed with regard to gestational age (overall mean 39.7 weeks, $p = 0.49$) and parity (overall mean 1.38 live births, $p = 0.56$).

Table 1 Length, weight, Apgar score at 1 and 5 min, free carnitine (fC0) from the newborn screening programme and fC0 from the voluntary screening programme compared between patients with PCD, patients born to mothers with PCD and controls

		n	Length cm	Weight gram	Apgar 1 min	Apgar 5 min	fCO newborn scr. µmol/L	fCO voluntary scr. µmol/L
Patients w. PCD	Male	44	53.9 (2.4)	3,727 (611)	9.1 (1.2)	9.8 (0.5)	7.9 (5.6)	3.6 (1.9)
	Female	35	52.3 (2.7)	3,462 (589)	9.2 (1.1)	9.9 (0.3)	8.4 (7.0)	3.1 (1.1)
	Total	79	53.2 (2.6)	3,612 (611)	9.2 (1.2)	9.9 (0.4)	8.1 (6.2)	3.4 (1.6)
Patients born to mothers w. PCD	Male	26	54.4 (2.4)	3,977 (599)	8.8 (1.7)	9.6 (1.0)	11.4 (6.0)	14.0 (2.6)
	Female	30	52.4 (3.1)	3,526 (638)	9.0 (1.4)	9.9 (0.3)	11.8 (6.8)	12.3 (3.5)
	Total	56	53.3 (3.0)	3,726 (654)	8.9 (1.5)	9.7 (0.8)	11.6 (6.3)	13.0 (3.2)
Controls	Male	166	53.6 (2.1)	3,796 (515)	9.5 (0.9)	9.9 (0.3)	44.1 (22.3)	22.2 (5.8)
	Female	147	52.7 (2.1)	3,627 (517)	9.1 (1.7)	9.8 (0.9)	43.8 (19.6)	20.8 (5.4)
	Total	313	53.2 (2.1)	3,717 (522)	9.3 (1.3)	9.9 (0.7)	43.9 (21.0)	21.5 (5.6)

Mean (SD). Note that fC0 was determined in plasma in the newborn screening prg., while in whole blood in the voluntary screening prg.

Table 2 Three different newborn screening strategies during different time periods

Period	Years *n*	A PCD patients in total *n*	B PCD patients included in the newborn screening prog. *n*	C Patients found by newborn screening *n*	C in percentage of B %
1986–2001	16	35	27	23	85.2
2002–2008	7	19	8	2	25
2009–2014	6	9[a]	5[b]	5	100

[a] The total number of patients is lower than expected, likely due to a lack of detection of patients with other genotypes than c.95A>G/c.95A>G
[b] The four remaining newborns had received L-carnitine before the blood test

Apgar

There was no significant difference between the groups in Apgar scores at 1 ($p = 0.18$) and 5 min ($p = 0.48$) (Table 1). Apgar scores at 1 min ranged from 5 to 10 in newborns with PCD and newborns born to mothers with PCD compared to 1 to 10 in controls. Apgar scores at 5 min ranged from 8 to 10 in newborns with PCD, 7 to 10 in newborns born to mothers with PCD and 3 to 10 in controls.

Free Carnitine, fC0

There was a significant difference in mean fC0 between controls and the other groups in both the newborn screening and the voluntary screening programmes ($p < 0.01$) (Table 1). There was though no significant difference in mean fC0 between newborns with PCD and newborns born to mothers with PCD measured in the newborn screening programme ($p = 0.34$) – while fC0 measured in the same groups later in the voluntary screening programme differed significantly ($p < 0.01$) (Table 1).

1986–2001, Retrospective Screening

A total of 35 PCD patients were born in the period 1986–2001 – of whom 27 (77%) had neonatal DBS samples available in the biobank. Twenty-three of the 27 PCD patients were identified by the retrospective screening of the stored DBS (85.2%) (Table 2).

2002–2008, Pilot Project

Nineteen PCD patients were born during the pilot project from 2002 to 2008, but only two of the eight patients, who participated in the pilot extended newborn screening programme, were identified by the screening algorithm used at that time (25%) (Table 2).

2009–2014, Routine Newborn Screening

Nine PCD patients were diagnosed during 2009–2014, of whom all five patients, who had not already received L-carnitine supplementation before the DBS was taken, had fC0 below the cut-off level and were thus screen positive for PCD (Table 2).

Table 3 Comparing the three different newborn screening strategies with regards to detection of c.95A>G homozygous newborns

Period	Years *n*	A c.95A>G/C.95A>G patients in total *n*	B c.95A>G/c.95A>G patients included in the newborn screening prog. *n*	C c.95A>G/c.95A>G patients found by newborn screening *n*	C in percentage of B %
1986–2001	16	16	12	12	100
2002–2008	7	12	5	1	20
2009–2014	6	8	4[a]	4	100

[a] The four remaining newborns had received L-carnitine before the blood test

c.95A>G Homozygotes

The retrospective screening of the stored DBS samples identified all known 12 patients homozygous for the severe c.95A>G mutation born between 1986 and 2001 (Table 3). However the pilot newborn screening programme only uncovered one of five known c.95A>G homozygous patients born in the period 2002–2008 (20%) (Table 3). In the current screening programme all four patients homozygous for the c.95A>G mutation born between 2009 and 2014, who had not received L-carnitine prior to the blood test being taken, were revealed (100%) (Table 3).

Discussion

We have shown that length, weight and Apgar scores at birth are not significantly affected in newborns with PCD and newborns born to mothers with PCD compared to controls. Newborns with PCD and newborns born to mothers with PCD seem to have a normal foetal development and respond and function normally at birth, even though their carnitine levels are low. Symptoms of cardiomyopathy and metabolic decompensation seem to develop beyond neonatal age in those children with PCD, who develop symptoms (Stanley 2004; Magoulas and El-Hattab 2012).

Carnitine Levels in Newborns

Our study showed that mean fC0 levels in newborns with PCD and newborns (PCD carriers) born to mothers with PCD did not differ a few days after birth (Table 1) – but when measured later in the voluntary screening programme there was a significant difference. Our data indicate that maternal carnitine levels during pregnancy significantly reduce the level of fC0 in newborns, as was also demonstrated by Novak et al. in the 1980s, who showed a direct correlation between maternal and foetal carnitine levels (Novak et al. 1981). Pasquali and Longo also showed that carnitine levels measured within 2 days from birth in infants with PCD fell when measured again after 14 days while carnitine levels in infants born to mothers with PCD increased after 14 days (Pasquali and Longo 2013).

Screening Programmes

It is remarkable that the retrospective screening programme (1986–2001) identified 85.2% of all PCD patients and 100% of the patients homozygous for the severe c.95A>G mutation while the pilot screening programme (2002–2008) only uncovered 25% and 20%, respectively (Tables 2 and 3). The reason lies in the screening algorithm for PCD used in the pilot screening programme between 2002 and 2008. Instead of looking only for a low level of fC0 to find potential PCD patients, the algorithm also required that acetyl-carnitine C2 was below a certain cut-off. This proved to be wrong because retrospective data analysis shows that if only the fC0 cut-off had been used in the algorithm then the pilot screening programme (2002–2008) would have uncovered seven out of eight PCD patients and all five c.95A>G homozygous patients. The bulk of known PCD patients were diagnosed through the population-based voluntary screening programme established in 2009 (Rasmussen et al. 2014b).

Differing Genotype Detection

Although the rate of detection in the newborn screening programme between 2009 and 2014 seems to be complete it is remarkable that all but one newborn with PCD diagnosed during the period was homozygous for the severe c.95A>G mutation (Tables 2 and 3). Thus compared to genotype distribution seen in the voluntary screening programme, there seems to be a reduced detection of subjects with other genotypes than c.95A>G/c.95A>G (Rasmussen et al. 2014c). The patients homozygous for the severe c.95A>G mutation have the lowest fC0 levels of all Faroese PCD patients, which is likely why they seem to be better detected

in the current screening programme (Rasmussen et al. 2014c). The retrospective screening programme based on DBS samples from 1986 to 2001 seems to have been more sensitive with regard to identifying other genotypes as well (Table 2). This might be because the lower cut-off level was set higher than absolutely needed to compensate for a higher mean fC0 because of acylcarnitine degradation over time. The reason could also be the fact that the maternal influence on fC0 measured in newborns was weaker before 2009 due to later blood sampling after birth, 4–9 days versus the current 2–3 days. Furthermore the highly increased public attention concerning PCD in the Faroe Islands since 2009 has led to the use at an unknown extent of self-administered over-the-counter supplements with L-carnitine by pregnant women, which may increase the neonatal level of fC0 and thus make the current neonatal screening for PCD less efficient.

Improve Detection in the Faroe Islands

Increasing the lower cut-off value of fC0 would likely increase the sensitivity of the current neonatal screening programme, but it would also increase the number of false positives and would probably not ensure a full detection of PCD patients because of the strong maternal influence on fC0 levels at the time of birth (Strnadova et al. 2007; Fingerhut et al. 2009). Instead a strategy of either an immediate repeated test of a new DBS if the screening result was borderline or an additional routine screening test with measurement of unbutylated fC0 at, e.g., 4 weeks after birth could be proposed (Longo et al. 2006). A third option is genetic screening for known PCD-related mutations in all Faroese newborns. Considering the high prevalence and public focus on PCD with a high likelihood of unprescribed supplementation with L-carnitine in the Faroe Islands the latter or third option would be the most obvious to ensure the highest possible detection of PCD in newborns.

Conclusion

Newborns with PCD and newborns born to mothers with PCD do not differ in length, weight and Apgar scores compared to controls. Current neonatal screening for PCD with testing only 2–3 days after birth in the Faroe Islands does not capture all patients with PCD that may benefit from preventive treatment. A strategy of a second screening a few weeks after birth measuring unbutylated free carnitine or genetic screening for mutations in the *SLC22A5* gene may be advantageous.

Acknowledgements The authors would like to thank the laboratory in the National Hospital in the Faroe Islands.

Compliance with Ethics Guidelines

Conflict of Interest

Jan Rasmussen, David M. Hougaard, Noreen Sandhu, Katrine Fjællegaard, Poula R. Petersen, Ulrike Steuerwald and Allan M. Lund all declare that they have no conflict of interest.

Contribution of Individual Authors

Jan Rasmussen: First author and responsible for planning, conducting and reporting the work in the chapter.

David M. Hougaard: Second author and responsible for planning and critically reviewing the work in the chapter.

Noreen Sandhu: Third author and responsible for planning, conducting and critically reviewing the work in the chapter.

Katrine Fjællegaard: Fourth author and responsible for planning, conducting and critically reviewing the work in the chapter.

Poula R. Petersen: Fifth author and responsible for conducting and critically reviewing the work in the chapter.

Ulrike Steuerwald: Sixth author and responsible for planning and critically reviewing the work in the chapter.

Allan M. Lund: Last author and responsible for planning, conducting and critically reviewing the work in the chapter.

Take-Home Message

Primary carnitine deficiency does not affect the health status of newborns and free carnitine should be measured when performing newborn screening.

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