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Medium Chain Acyl-CoA Dehydrogenase Deficiency: 3 years of Newborn Screening

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Abstract

Aims

Ireland added Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) to the National Newborn Bloodspot Screening Programme (NNBSP) in December 2018. The aim of this study was to reassess the incidence of MCADD in Ireland since screening began and characterise cases biochemically, clinically and genetically.

Methods

Data were retrospectively obtained from the NNBSP laboratory database from 03/12/2018 to 03/12/2021, including number of infants screened, total positive screens and true positive screens, and corresponding biochemical data. Infants with confirmed MCADD had their medical notes examined for clinical outcomes, family history and genetics.

Results

11 patients had true positive screens for MCADD, giving an estimated incidence of 1 in 16,164. All patients had at least one c.985A>G variant in *ACADM*. Screening C8/C10 ratio values were highest in homozygotes for c.985A>G and compound heterozygotes for c.799G>A/c.985A>G. One infant presented with hypoglycaemia prior to screening results being available. Two had hypoglycaemia related to comorbid diagnoses. No patients have decompensated since diagnosis. One additional

case was detected through familial cascade screening. Mild intermittent elevations in creatine kinase were noted in several patients, without symptoms.

Discussion
The incidence of MCADD in Ireland is higher than previous estimates. Screening has resulted in positive outcomes and the biochemical, genetic and clinical profile of patients is in keeping with expectation.

Introduction

Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD, OMIM # 201450) is the most common disorder of fatty acid oxidation worldwide[1]. It is an autosomal recessive disorder caused by pathogenic variants in the *ACADM* gene which results in deficiency of the medium chain acyl-CoA dehydrogenase enzyme, required for metabolising medium chain fatty acids and providing substrate for formation of ketone bodies[2]. Patients with MCADD present clinically in acute metabolic crisis with hypoketotic hypoglycaemia and encephalopathy at times of reduced glucose intake or increased catabolism[2].

Ireland added MCADD to the National Newborn Bloodspot Screening Programme (NNBSP) in December 2018[3]. A study of the incidence of MCADD prior to initiation of screening suggested an incidence of 1:71,650 children in Ireland, with a prevalence of 1.23 per 100,000 children[4]. Another unpublished study which screened newborn screening cards for the most common pathogenic variant in *ACADM* suggested an incidence of 1:66,000 births[4]. In Ireland in 2019 there were 59,796 births[5], therefore it could be expected that approximately one child per year will have a true positive newborn screen for MCADD.

MCADD deficiency is a highly treatable disorder. Limitation of fasting times, and ensuring adequate glucose intake during times of metabolic stress such as infection are the mainstay of management[2]. There is significant risk of morbidity and mortality in undiagnosed patients whose first presentation is symptomatic, which can be mostly prevented by newborn screening[6].

We aim to review data available since the commencement of newborn screening for MCADD deficiency in Ireland to characterise the confirmed cases biochemically, clinically and genetically, and reassess the incidence of MCADD in Ireland in light of the data available.

Methods

The NNBSP in Ireland collects dried blood spot samples from infants between 72 and 120 hours of life, irrespective of prematurity, feed status or clinical condition. Samples are air-dried and sent to a central laboratory for processing. If a red blood cell transfusion is due to be given prior to 72 hours of life, a pre-transfusion sample is also obtained. Premature infants, low birth weight infants, and infants not established on full feeds and/or on total parenteral nutrition (TPN) at time of first sampling may have further samples collected until the baby has been established on full feeds for at least 24 hours. Informed consent is obtained before collection of samples.

The C8, C10 and C2 acylcarnitines are assayed using tandem mass spectrometry. The assays are underivatized. Where the C8 measurement is ≥0.16umol/l, the measurements are repeated in duplicate. If the C8 average in triplicate (or 2/3 results) is ≥0.16umol/l then the C8/C10 and C8/C2 ratios are determined for each spot. If the C8/C10 average (or 2/3 results) is ≥1.5, the screen is considered positive and MCADD is suspected. If the initial C8 measurement is ≥1umol/l, the assays are repeated in duplicate and if the average of the triplicate (or 2/3 results) is ≥1umol/l, the screen is considered positive and MCADD is suspected. These cut off values are revised periodically and the values in the above algorithm may have been occasionally adjusted during the study period to increase specificity and sensitivity of screening.

Confirmatory testing is carried out comprising a second dried bloodspot card for acylcarnitines using a derivatised method, and a urine for organic acid analysis. Genetic testing of *ACADM* is carried out in all cases where the biochemistry is diagnostic.

Data were obtained from the NNBSP database covering the period 03/12/2018 (beginning of screening) to 03/12/2021. Data gathered included total number of infants screened, total number of positive screens and total number of true positive screens, and corresponding biochemical data. The infants with true positive screens were identified and their clinical medical notes were examined to assess clinical outcomes, family history and genetic test results. Patients were assigned a study number and all data were anonymised.

Statistical analysis (Microsoft Excel) of the C8 values was performed using a two sample t-test assuming unequal variances.

Ethical approval was obtained from the hospital research ethics committee for this study (Reference 21.012).

Results

The total number of infants screened during the study period was 177,800. This number represents the total number of first screening cards obtained, and does not include repeat screens. Of the total

screens performed, 55 were considered positive. Based on further data gathered by the newborn screening laboratory, 37 of these were deemed false positives related to ongoing administration of total parenteral nutrition (TPN) to the infant. All of these infants were born preterm (all <33 weeks gestation). Seven further cases were also deemed false positives - two patients who had previously received TPN, one infant receiving a medium chain triglyceride (MCT)-based infant formula, one poor quality/expired newborn screening card, and in two cases no reason was noted on the database for the false positive result. One additional infant was found to be a carrier for a pathogenic variant in the *ACADM* gene, with no second variant identified. In total, 44 screens (80% of all positive screens) were false positives.

There were eleven true positive cases (20%). All confirmed cases were delivered at term. One case was detected in 2018, immediately after the commencement of screening. Two cases were detected in 2019, two in 2020 and six in 2021 (up to the end of the study period). Based on the total number of first screening cards obtained during the study period, this gives a positivity rate of 1 in 16,164.

Demographic details of the confirmed cases are summarised in Table 1. In one case the first screening card was taken late at 161 hours of life. In two cases the first screening card was taken early at 21 hours and 15 hours of life – in both of these cases the C8 was elevated on first screening card and both cases were siblings of known patients with MCADD.

All MCADD cases had a C8 of >1umol/l measured on their first screening card, with a median value of 3.88umol/l (mean 6.2umol/l, range 1.05-22.7umol/l)). Comparing this to the false positive cases, the highest C8 value on first positive screening card was 0.46umol/l with a median value of 0.19umol/l (mean 0.22umol/l, range 0.14-0.46umol/l)**.** The difference in mean C8 values on first screening card between the true positive and false positive cases, was found to be statistically significant ($p < 0.02$).

Patient ID	Sex	Ethnicity	Age (hours) at time of 1st screening card	Age (hours) at time of 2nd screening card
1	F	Irish	96	N/A
2	F	Irish	90	144
3	M	Irish	72	187
4	F	Irish	117	197
5	F	Irish	116	161
6	M	Irish Traveller	89	N/A
7	F	Irish/British	72	N/A
8	F	Irish	74	177
9	M	Polish	161	208
10	M	Irish	21	146
11	M	Roma	15	Not recorded

Table 1: Patient Demographics.

All patients had biochemical confirmation of the diagnosis made on dried blood spot acylcarnitine profile (ACP) and urine organic acid (UOA) analysis. All patients had variable elevations of hexanoylglycine, suberylglycine and dicarboxylic acids on UOA analysis. One patient's confirmatory ACP showed slightly raised medium chain acylcarnitine species with a normal C8:C10 ratio, and UOA analysis showed only hexanoylglycine, suggestive of a mild biochemical MCADD phenotype. ACP findings are summarised in table 2.

Five patients in the cohort had intermittent mild elevation of creatine kinase (CK) levels despite remaining clinically asymptomatic and not necessarily correlating to intercurrent illness.

Patient ID	Mean C8 NBS (umol/l)	Mean C8/C10 NBS	ACP C8 confirmation (umol/l)	ACP C8/C10 confirmation	Genotype	Hypoglycaemic events
1	1.05	2.54	1.31	2	c.985A>G/c.199T>C	None
2	2.88	8.13	2.53	7.23	c.985A>G/c.799G>A	None
3	0.73	1.17	0.41	0.87	c.985A>G/c.127G>A	None
4	1.52	7.51	1.72	3.58	c.985A>G/c.799G>A	None
5	4.73	11.91	6.13	9.1	c.985A>G/c.985A>G	None
6	3.89	12.07	20.82	11.7	c.985A>G/c.985A>G	One
7	3.85	7.92	1.63	7.5	c.985A>G/c.999_1011dup	None
8	5.3	16.7	2.54	11.5	c.985A>G/c.799G>A	None
9	1.11	11.27	1.51	11.6	c.985A>G/c.985A>G	Three*
10	13.84	14.15	24.17	13.1	c.985A>G/c.985A>G	None
11	9.78	11.66	20.5	10.7	c.985A>G/c.985A>G	Multiple*

Table 2: Biochemical, genetic and clinical correlation. NBS = Newborn screening, ACP = dried bloodspot acylcarnitine profile. Reference range for C8 on ACP is 0-0.26umol/l in neonates up to 2 weeks. Asterisk denotes hypoglycaemia unrelated to MCADD diagnosis. All genotypes reported using reference sequence NM_000016.6.

All patients had genetic testing performed at diagnosis for pathogenic variants in *ACADM*. The most common pathogenic variant identified in the *ACADM* gene was the c.985A>G (p. Lys329Glu) variant, present in the homozygous state in five patients and in the compound heterozygous state in the other six. The genetic data is summarised in table 2. Three of the patients had high-risk newborn screening in the context of family history of MCADD. Of note, one infant had a sibling who died in

the first days of life with MCADD, prior to the introduction of screening. Familial cascade testing was performed where appropriate and the older sibling of one child was found to also have MCADD. The sibling was well and had never presented clinically.

Patient 6 presented clinically on day three of life prior to the newborn screening result being available. There was a history of poor feeding at home followed by a cyanotic event for which the patient was brought to the emergency department of the local hospital. Feeding had been exclusively with infant formula. Point of care blood glucose at presentation was noted to be low at 2.2mmol/l and a biochemical hypoglycaemia workup was performed, including ACP. The patient was managed with intravenous dextrose infusion and supplementary nasogastric tube feeding until adequate oral feed volumes were established. The newborn screening result became available on day **two** of his admission and the diagnosis of MCADD was confirmed on the previously obtained acylcarnitine profile. This patient is homozygous for the classical c.985A>G variant.

Patient 9 had three episodes of hypoglycaemia in the immediate newborn period. Biochemical sampling revealed hyperinsulinism in addition to his positive newborn screen for MCADD and the hypoglycaemia was felt to be related to transient hyperinsulinism. Patient 11 had many episodes of hypoglycaemia with elevated lactates prompting further investigation and has been found to have a comorbid diagnosis of glycogen storage disease (GSD) type 1b.

All other patients in the cohort have had a clinically unremarkable course. None have had a significant episode of metabolic decompensation, despite a number of typical childhood infections. Eight of the eleven patients are currently on carnitine supplementation. One patient has speech delay and the rest are developmentally appropriate for their age.

Discussion

In this study, we found an incidence of 1 in 16,164 when screening for MCADD in the newborn period. Previous estimates of the incidence of MCADD in Ireland range from 1 in 66,000 to 1 in 71,650[4]. We predict that the true incidence of MCADD in Ireland is considerably higher, which would be in keeping with previous studies which have shown that the estimated prevalence of MCADD from newborn screening is at least double that of estimated prevalence from clinically detected cases[7].

Not all babies with MCADD will be detected by newborn screening. Patients carrying the c.199T>C variant in combination with another pathogenic variant have been shown to have lower acylcarnitine markers compared to other genotypes, and there is a potential for false negative screening result[8]. Additionally, some patients will present clinically prior to their newborn screen being completed (as happened with one patient in our cohort), and some families will refuse newborn screening. Infants with very low free carnitine levels may have low C8 measurements due to carnitine depletion and may not be detected by the newborn screening programme[2]. We are not aware of any missed cases during the screening period, however these cases may not present clinically, and even in the event of such a case presenting with hypoglycaemia, a diagnostic workup may not be performed.

The common c.985A>G pathogenic variant in the *ACADM* gene causes the replacement of a lysine by a glutamate at position 329 of the precursor protein (p.K329E). This accounts for approximately 80% of clinically apparent disease in most of Western Europe[9]. This is reflected in the genetic profile of patients identified by our newborn screening programme, who all were at least heterozygous for this variant. The only patient in our cohort who has had a clinical presentation with hypoglycaemia that was felt to be related to the diagnosis of MCADD is homozygous for this common variant, which is in keeping with previously published data on the potential severity of the phenotype[10,11].

The highest values for C8 and C8/C10 ratio were obtained in the patients who are homozygous for c.985A>G and compound heterozygous for c.985A>G/c.799G>A. The values for C8 on confirmatory ACP were >20umol/l in three patients, all homozygous c.985A>G, presumably due to the timing of sampling in two of the patients (6 and 11) who both had episodes of symptomatic hypoglycaemia.

The lowest values on both the screening card and subsequent confirmatory sampling were obtained in Patient 1, corresponding to the genotype c.985A>G/c.199T>C, and Patient 3, corresponding to the genotype c.985A>G/c.127G>A. This is in keeping with previously published data that both of these genotypes are associated with lower production of biochemical markers[9,10]. The variant c.127G>A has never been reported to cause a clinical phenotype[12].

The majority of false positive cases were due to TPN use. Many neonatal units in Ireland use standardised PN incorporating SMOFlipid, which has a high levels of medium chain triglycerides. This has been reported to cause false positive newborn screening results for MCADD[13]. To date, C8

levels in false positive cases are lower than in true positive cases, without overlap. There is future potential to consider raising the cut off values to reduce the number of false positives, however at this time we recommend maintaining current cut off values to avoid missed cases while further data is gathered to support this.

Interestingly, patient 3 had persistent elevation of liver transaminases and CK levels during the first year of life, despite never presenting with a clinical decompensation. These findings have now normalised and his acylcarnitine profile has been normal on serial analysis. Several other patients have also had intermittently raised CK levels which do not appear to correlate with any clinical symptoms or intercurrent illness.

There were more new cases diagnosed by newborn screening in 2021 than in previous years, and this may reflect the birth of a number of siblings of known MCADD patients during this time period (three out of the six new cases).

In conclusion, the biochemical and clinical phenotype of patients diagnosed with MCADD since the introduction of newborn screening largely is in keeping with expectation, however the incidence has increased. Further analysis is warranted in a few years to calculate the true incidence of MCADD in Ireland.

Conflict of Interest:
None declared.

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