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Biochemical and molecular characteristics of patients with organic acidaemias and urea cycle disorders identified through newborn screening

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ABSTRACT

Background: In recent years it has become clear that newborn screening (NBS) programmes using tandem mass spectrometry identify "patients" with "classical" inborn errors of metabolism who are asymptomatic. This observation raises issues regarding medicalization of "non-diseases," potentially unnecessary treatment and unnecessary anxiety to parents.
Aims: This study aims to identify possible markers that may assist in predicting the need for treatment of infants with "classical" organic acidaemias (OA) and urea cycle disorders (UCD) diagnosed through NBS.
Methods: Medical records of all patients with classical OA and UCD detected through the Victorian NBS programme from February 2002 to January 2014, or diagnosed clinically between 1990 and January 2002 were retrospectively reviewed.
Results: Neonatal presentation did not always predict the need for on-going strict treatment. Blood concentrations of amino acids and acyl-carnitines and the changes thereof in follow-up samples correlated with severity in citrullinaemia-I, possibly isovaleric acidaemia but not in argininosuccinic aciduria or propionic acidaemia. Some specific mutations correlate with "attenuated" citrullinaemia-I. Gender may affect clinical outcome in propionic acidaemia.
Conclusions: Changes in blood concentration of certain metabolites (amino acids, acyl-carnitines) in the first weeks of life may be predictive of the need for treatment in some disorders but not in others. Mutation analysis may be predictive in some disorders but whether or not this should be considered as second-tier testing in NBS should be discussed separately.

1. Background

Inborn errors of metabolism (IEM) are a major cause of morbidity [1] and mortality [2] and therefore present a significant public health problem. The natural history of many of the inborn errors of intermediary metabolism is well characterised and treatment strategies have been in place for years. As such, these disorders are suitable candidates for newborn screening (NBS) according to the widely accepted Wilson and Jungner criteria for disease screening [3]. In recent years, screening programmes using tandem mass spectrometry (TMS) have replaced and extended previously used methodologies. This technology enables a quantitative analysis of multiple amino acids and acyl-carnitines in dried blood spots and efficiently detects multiple IEM in one single quick test [4,5].

Detecting patients through NBS seems to have clear benefits; however, it is clear now that more "patients" are identified in comparison with clinically identified patients in unscreened populations [6–9]. The identification of infants who remain asymptomatic raises issues regarding medicalization of "non-diseases," potentially unnecessary treatment including dietary restriction [10,11], medications and "prophylactic" admissions, parental anxiety [12], and overall increased costs to the health system. Attempting to predict the need for on-going monitoring and treatment and the prognosis of a diagnosed infant would therefore be desirable but may prove difficult.

In this study we aimed to identify early predictive factors of disease severity in infants diagnosed with "classical" organic acidaemias (OA)

Abbreviations: NBS, newborn screening; TMS, tandem mass spectrometry; DBS, dried blood spots; OA, organic acidaemias; PA, propionic acidaemia; IVA, isovaleric acidaemia; MMA, methylmalonic acidaemia; UCD, urea cycle disorders; CIT-I, argininosuccinate synthetase deficiency (citrullinaemia type I); ASA, argininosuccinate lyase deficiency or argininosuccinic aciduria.

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and urea cycle defects (UCD) that are reliably identified through NBS. Acknowledging our relatively small population and patient cohort, we attempted to also rely on previously published reports that may shed light on this issue.

2. Methods

The NBS laboratory has been screening newborns in Victoria, Australia using TMS since February 2002 with 99.4% coverage [13]. Testing is performed on dried blood spots (DBS) obtained at 48–72 hours of age. Newborns picked up by the screening test undergo further confirmatory tests by means of repeat acyl-carnitine profile, urine organic acids analysis and orotic acid level, urine and plasma amino acids analysis and plasma ammonia, as well as enzymatic and molecular testing, as deemed appropriate. A centralised clinical metabolic service that works closely with the Newborn Screening Laboratory provides follow-up and treatment to all patients with IEM in the state. Regardless of whether the infant is clinically diagnosed or through NBS, "alert," initiation of contact with the hospital, admissions and treatment protocols are identical once the child is diagnosed.

Infants with the following organic acidaemias were included: methyl malonic acidaemia (methylmalonyl-CoA mutase deficiency [MMA] and excluding cobalamin defects), propionic acidaemia (PA), isovaleric acidaemia (IVA). Infants with the urea cycle disorders included were those with argininosuccinate synthetase deficiency (citrullinaemia type I, CIT1) and argininosuccinate lyase deficiency (argininosuccinic aciduria, ASA). The incidence of each disorder was calculated relative to the total number of newborns screened from February 2002 through January 2014. Given the rarity of the disorders studied and the small number of patients, the Poisson distribution was used to calculate the 95% confidence interval (CI) of incidence of each disorder over 12 years of newborn screening (http://statpages.org/).

We reviewed all health records and laboratory records of all patients with these disorders, detected through newborn screening from February 2002 through to the end of January 2014 and all records of all patients with these disorders diagnosed following a clinical presentation in the 12 years preceding the expansion of the NBS programme. Details regarding treatment, follow-up and hospital admissions were noted. Alert guidelines have been similar for patients diagnosed following a clinical presentation and those diagnosed following NBS and decisions regarding prophylactic admissions are similar. Patients were considered asymptomatic if they had a confirmed inborn error of metabolism without showing any metabolic derangement or known disease specific symptom during the longest follow-up period possible.

The study was approved by the Ethics Committee of the Royal Children's Hospital, Melbourne, Australia (HREC 32088A).

3. Results

A total of 847,418 newborns were screened over 12 years, ranging from 62,384 to 77,699 per year. Altogether, 29 patients with classical organic acidaemias and urea cycle disorders detected through the expanded NBS programme were diagnosed: six patients were identified with IVA, six with PA, one with MMA. Eight patients were diagnosed with CIT-I and eight patients with ASA. Diagnosis was confirmed by measuring organic acids in urine (patients with OA), amino acids in urine and plasma (patients with UCD), enzyme analysis (one patient with PA) or mutation analysis (11 patients). No children with normal NBS results were subsequently diagnosed clinically with any of these IEM. Infants were of diverse ethnicity, with five being of consanguineous families. A total of 136 hospital admissions occurred in the cohort up to age 2.5 years. Details regarding the type of admission (precautionary measure during intercurrent illnesses or metabolic decompensation) are presented in Table 1.

Six infants were diagnosed with IVA (incidence 1:128304, 95% CI 1:59217–1:384913). C5-carnitine levels on their first newborn screening sample ranged from 3.7 to 4.39 μmol/L (cutoff 0.85). One patient (IVA-3) was symptomatic in the first days of life but had no symptoms thereafter (19 m follow-up). None of the other patients had any metabolic derangements or symptoms so far. Growth parameters and motor and cognitive development are normal in all children. Two patients were diagnosed with IVA following a clinical presentation in the 12 years prior to the expanded NBS: one presented in the first days of life and deceased shortly thereafter; the other was diagnosed at age of 3 years following an intercurrent illness and is autistic.

PA was diagnosed in six infants (incidence 1:128304, 95% CI 1:59217–1:384913, four male infants) whose C3 carnitine levels ranged from 5.7 to 47.8 μmol/L (cutoff 8.3) in the first screening sample. Three patients had mutation analysis (Table 2). The follow-up time varied from 15 m to 8y/11 m. Clinical manifestations were variable: three infants (all male) had metabolic decompensation in the neonatal period. Another male patient presented rather acutely with severe movement disorder and hypotonia at age 4 m with no overt metabolic decompensation. He had one episode of acute metabolic acidosis, at age of 6 months, but no further episodes thereafter (current age, 8 years/11 months). Growth parameters of all children are adequate, except for one of the symptomatic patients, who has end-stage renal failure following nephrotic syndrome due to C1q deposits (focal segmental glomerulosclerosis at age of 2 years/6 months). The two female patients are metabolically asymptomatic, including at times of intercurrent illnesses. Liver function is normal in all patients. Of the total 57 admissions during the first 2 years of life (46 and 11 in male and female patients, respectively), 19 were due to metabolic decompensation

Table 1
Patients' admissions to hospital.

	PA	IVA	MMA	CIT I	ASA
Number of patients	6	6	1	8	8
Sex: male (*n*)	4	4	1	5	1
Follow-up time	2 years–9 years/7 months	2 years–9 years/9 months	11 years/6 months	1 years/1 month–8 years/10 months	5 months–9 years
Overall admissions[a]	Male 46/female 11	11	11	15	10
Overall admissions due to metabolic decompensation:	Male 18/female 2	1	1	1	1
Overall admissions: 1st year of life	Male 29/female 2	3	1	8	5
1st year of life admissions due to metabolic decompensation:	Male 9[b]/female 1[c]	1[c]	1[c]	1[c]	1[c]
Overall admissions: 2nd year of life	Male 17/female 9	3	2	2	2
2nd year of life admissions due to metabolic decompensation:	Male 9/female 0	0	0	0	0

Y = year; M = month.
[a] Number of admissions in PA only in the first 2 years. Patient PA-5 has had multiple admissions for haemodialysis due to renal failure since then.
[b] Admission in the first 5 days after birth in four patients.
[c] Admission in the first 5 days after birth.

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