



Short Communication

Incidence of maple syrup urine disease, propionic acidemia, and methylmalonic aciduria from newborn screening data

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A B S T R A C T

Incidence for the branched-chain intoxication-type disorders, maple syrup urine disease, propionic acidemia and methylmalonic aciduria is dependent on the population screened. Here newborn screening results from three world regions, state screening laboratories in the United States, a region in Germany and Kuwait provides new incidence numbers. Maple syrup urine disease incidence in the United States was calculated to be 1: 220219, in South-West Germany 1: 119573 (Germany nationwide 1:177978), and in Kuwait 1: 59426. Incidence of propionic acidemia alone is calculated to be 1: 242741 in the United States, 1: 284450 in South-West Germany (Germany nationwide 1:202617) and 1:59426 in Kuwait. Incidence of isolated methylmalonic aciduria alone is 1:69354 in the United States, 1:568901 in South-West Germany (Germany nationwide 1:159199) and 1: 19809 in Kuwait. In the United States several newborn screening laboratories combine their results for propionic acidemia and methylmalonic aciduria, and also include combined remethylation disorders in the respective category, resulting in an incidence of 1:50709. Combined evaluation of methylmalonic aciduria, propionic aciduria and combined remethylation disorders results in a similar incidence for Germany of 1:67539. This evaluation of newborn screening incidences reflects some population differences for three intoxication-type metabolic disorders. However, different sample sizes of the populations screened over different time periods, and differences in case definitions for methylmalonic acidurias have to be considered when interpreting these data.

1. Introduction

The intoxication-type branched chain inherited metabolic disorders, maple syrup urine disease, propionic acidemia and methylmalonic aciduria can be life-threatening if left untreated. Newborn screening (NBS) has been used to identify individuals with a number of inherited metabolic disorders with the goal that early treatment prior to symptoms will improve outcomes. Moreover, several studies have shown NBS improves outcomes [1–4]. NBS results also provide an opportunity to look at incidence across several populations which can be useful to understanding their differences, but also limited by the manner the information is reported. Here we look at incidences of three branched chain intoxication type disorders, maple syrup urine disease, propionic acidemia and methylmalonic aciduria based on newborn screening data from three different countries.

NBS has gone through continuous improvement and mild protocol changes ever since the addition of tandem-mass spectrometry (tandem-MS) techniques. These improvements have led to changes in cut-offs and data analysis to decrease false positives and increased sensitivity.

Maple syrup urine disease (MSUD, MIM #248600) is characterized by branched chain ketoacid dehydrogenase dysfunction resulting in elevations of the branched chain amino acids, leucine, isoleucine, and valine as well as the diagnostic marker alloisoleucine. Clinically, patients have elevation in leucine during metabolic stress which is thought to increase cerebral edema leading to an intoxication-like phenotype [5]. MSUD has an overall incidence proposed to be about 1:185,000. MSUD (classical and occasionally intermediate) is now able to be identified by the presence of elevated leucine [6,7] or leucine + (usually sum of leucine, isoleucine and alloisoleucine) using dried blood on filter paper cards. This technique is used for NBS alone in some locations. Other locals currently also incorporate ratios (i.e. leucine/phenylalanine and leucine/alanine) to improve accuracy [8]. There is an increase incidence in some populations due to founder effects, including those seen in the Mennonite population (incidence of as high as 1:358 in the Old Order Amish) [2,9], in the Galician population in Spain (1 in 52,541), [10] and in the Ashkenazi Jewish populations [11].

Propionic acidemia (PA, MIM #606054) is a disorder characterized by elevated propionic acid, 3-hydroxypropionate, methylcitrate and

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propionylcarnitine (C3) which often presents with metabolic acidosis and hyperammonemia [12,13]. It can be identified by elevated C3 on NBS. Some locales also have started to use C3 ratios (i.e. C3/C2 and C3/C16) to improve accuracy. True incidence in Europe is unknown [14]. Incidence in Western countries is estimated to be 1 in 50,000 to 1 in 500,000 [15]. In isolated areas much higher incidence is observed, for example certain tribes in Saudi Arabia have incidences as high as 1 in 2000 to 1 in 5000 [16] and certain Mennonite communities have higher incidence [17].

Methylmalonic aciduria (without homocystinuria, MMA, MIM #251000, #251100, #251110) is caused by three different enzyme deficiencies Methylmalonyl CoA mutase (MCM, MIM*609058; E.C. 5.4.99.2), Cobalamin A (MMAA;MIM *607481), or Cobalamin B (MMAB; MIM*607568; E.C. 2.5.1.17). MMA incidence in Europe is not well known [14]. However, in Western cultures the incidence is estimated to be 1 in 48,000 to 1 in 61,000 [18]. Elevation of C3 is seen on NBS.

Cobalamin (B12) is required for MCM function and so dysfunction in its processing both due to underlying functioning issues and/or due to diminished quantity can sometimes be found by NBS. The metabolic disorders, Cobalamin C and D deficiency can also result in positive screening for MMA using C3 as a primary marker. These are usually coupled with elevations in total homocysteine but often times homocysteine or methionine (which is low in these disorders) are not routinely included in newborn screening strategies.

We recognize that incidence of metabolic disorders are impacted by ascertainment bias, population frequencies, and screening methods and so set out to use publicly available newborn screen data to determine whether we could calculate the incidence of MSUD, MMA, and PA for several populations. We found that there is most certainly a difference in incidence across populations and so it is best to recognize the risk within the population treated. We also notice that different systems report the data differently, complicating the calculations for incidences.

2. Material and methods

Newborn screening results from three different countries, the United States (US), Germany, and Kuwait were collected and analyzed. Each of these countries has a different reporting structure with more or less additional information available about diagnosis as well as differences in definition of positive results.

Online reporting data was queried from the US data base from 1991 to 2000 for positive newborn screens (defined as diagnosed with the disease in question) and compared to the on-line reported birth rates per US state. Some states do not publish their data; this includes Virginia, Maryland, Pennsylvania, etc. We were able to identify reports from California (2001–2006), Kansas (2010), Massachusetts (2001–2006), Michigan (2007–2013), New Jersey (2010–2013), New York (2004–2014), North Carolina (2001–2006), Washington (2009–2013) and Wisconsin (2001–2006). In addition, National Summary data from reports from 1991 to 2000 were also used. All these states use a tandem-MS, blood filter paper card approach.

In the US, different states have different approaches to reporting, different cut-offs and differences in whether ratios are used. In addition, the reports differ in that some states report PA and MMA together (due to marker C3 being in common, e.g. Michigan and New York) and other states report PA and MMA separately (e.g. Washington, California, Kansas, Wisconsin, North Carolina and Massachusetts). Different states use different approaches to identifying positive screens and this has also changed over the time surveyed. Most states in this composite analysis screen at 24–48 h of life. Determining which state used which cut-off and other ratios when is beyond the scope of this paper. False positive test results are not available for all states in these databases and so it is beyond the scope of this report as well.

A similar data extraction was made for positive newborn screens in the Heidelberg screening laboratory, performing newborn screening for

the area of South-West Germany (currently about 135,000 newborns screened per year). Newborn screening Heidelberg implemented tandem-MS based newborn screening using blood filter paper cards in the course of a pilot study in the course of the year 1998 and therefore provided screening data for this region for MSUD from 1998 to 2014. From 2002 on tandem-MS screening was recommended for the whole of Germany. In 2005 a binding national screening panel under the use of tandem-MS was implemented for the whole county in Germany, which mandates screening for 12 metabolic disorders including MSUD, but no longer PA, MMA, or combined remethylation disorders (due to their low incidence in the German population). Therefore data on PA and MMA are provided for 1998–2004 only. Incidences for all conditions are provided with reference to the number of children screened for these conditions. In addition data from the national screening reports covering all of Germany are provided for the period of 2000–2014 (data on MSUD for all years except 2003, for PA and MMA for the years 2000–2002 and 2004) (reports from 2004 on are available from: <http://www.screening-dgns.de/reports.php>). The numbers reported for Germany include the cases found in South-West Germany for the respective years.

Calculations for Kuwait are based on published results from 2015 based on their initial experience of using a tandem-MS approach on blood filter paper cards. There are a number of private clinics and hospitals in Kuwait and these institutions are not obligated to report their findings. These results are limited to only the deliveries outside these private clinics and may not reflect the population at large.

3. Results

Incidences here were determined based on available data from public and from private databases.

3.1. Incidence from newborn screening in the United States

We found the US incidence of MSUD is lower (Table 1) than the incidence cited in The Metabolic & Molecular Bases of Inherited Disease [5]. PA is about as rare as expected given the various estimates (Table 1) and MMA both with and without cobalamin remethylation disorders are close to the cited incidences.

3.2. Incidence from newborn screening in Germany and Kuwait

German incidence for PA is close to that reported in the US data, but the incidence of MMA is much lower (Table 2). It is unclear whether this is a population difference or due to some ethnic variations. If the remethylation disorders are included, the incidence is closer to the US data.

Kuwait has low numbers of individuals screened and identified with disorders (Table 3). As a result, these estimates may not reflect the actual population incidence. This analysis shows higher incidence for all three branched-chain intoxication type disorders than the US and Germany.

Table 1

Overall summary table from US data. Solo refers to only that diagnosis included in these numbers.

Disorder screened	Number found with disorder	Number of births	Incidence
Maple Syrup Urine Disease	91	21,141,094	1/220,219
Propionic acidemia (solo)	12	2,912,901	1/242,741
Methylmalonic aciduria (solo)	42	2,912,901	1/69,354
MMA and PA together (and includes cobalamins)	147	7,544,243	1/50,709

Table 2
Summary table for the German NBS data with details about region and years included.

Disorder screened	Region (years reported)	Number with disorder	Number screened	Incidence
MSUD	South-West Germany (1998–2014)	14	1,674,021	1/119,573
MSUD	Germany (2000–2014) ^a	51	9,076,891	1/177,978
PA	South-West Germany (1998–2004)	2	568,901	1/284,450
PA	Germany (2000–2004) ^a	11	2,228,782	1/202,617
MMA	South-West Germany (1998–2004)	1	568,901	1/568,901
MMA including cobalamin C/D	South-West Germany (1998–2004)	5	568,901	1/113,780
MMA and PA together, and including cobalamin C/D	South-West Germany (1998–2004)	7	568,901	1/81,272
MMA	Germany (2000–2004) ^a	14	2,228,782	1/159,199
MMA and PA together, and including cobalamin C/D #	Germany (2000–2004) ^a	33	2,228,782	1/67,539

^a No data available for the year 2003; # including 6 cases with undifferentiated report of PA/MMA/Cbl from the year 2004 not included in the respective categories for Germany above.

Table 3
Summary table of the NBS data from Kuwait.

Disorder screened	Number with disorder	Number screened	Incidence
MSUD	1	59,426	1/59,426
PA	1	59,426	1/59,426
MMA	3	59,426	1/19,809

4. Discussion

Here we report three populations and their newborn screening results over different time periods to determine whether we can better characterize incidence or occurrence of three branched-chain intoxication type disorders. As is known, variation among individuals and heterogeneity of population changes incidence of disorders. Founder effects are common within isolates, but the incidence can be difficult to determine. There are some similarities in the results reported here, but also some important differences. Kuwaiti data shows a higher incidence/occurrence of MSUD, MMA and PA than either Germany or the United States. This may be a reflection of increased consanguinity within this population and small numbers.

One would suspect that affected individuals in Kuwait have several of the Arabian Peninsula founder mutations. Some of the more commonly reported mutations include: *PCCA*: p.G117D, p.V681_A706del26, *PCCB*: p.E331*, p.C381Y [19], *MUT*: p.R93H, p.Q37*, p.Y110C, p.Q734* [20], MSUD: *BCKDHA* p.N302A, *BCKDHB*: p.M1?, p.G66R, p.T273P, p.G335D, p.G336S [21].

Germany has a slightly higher incidence for MSUD and similar incidence of PA compared to the US. There are some more common *PCCB* mutations in the German/Austrian population including p.V205N, p.G406fs, p.R410W, and p.N563D. There does not appear to be founder mutations for the greater German population for MSUD.

The US mutation profile is variable due to its heterogeneous population. More commonly reported mutations include: the *BCKDHA* (Mennonite) mutation p.Y438N, *PCCA* mutation p.T62_S100Del39 and *PCCB* mutations p.G406 fs and p.R410W [19].

For immigrant rich areas and population isolates no matter the location, the frequency of seeing certain disorders reflects that of the founding population.

All US results reported here are using the tandem-MS technology. Improvement over the years has led to changes in cut-offs and addition of compound ratios which improve the sensitivity and decrease false positives. Most US states do not report their false positive rates and so this cannot be easily determined even with their improvements. Thus, positive and negative predictive values also vary over time.

When looking at these data, it is important to note that combined remethylation disorders like cobalamin C and D deficiency are not necessarily excluded from MMA data in all reporting systems, and so the reported incidence of isolated MMA may be slightly elevated from reality. The MMA data for Germany reported here exclude combined

remethylation disorders (Table 2, MMA from South-West Germany 1998–2004). For comparison with the US data, a combined incidence for MMA, PA and Cbl C/D has been reported for Germany (Table 2, last row, labeled with #). With 1/67,539 this is closer to the combined incidence reported for the US. Therefore also differences in the definition of confirmed diagnoses in different reporting systems have to be considered when interpreting data for incidence calculations as illustrated by the methylmalonic aciduria data shown here.

On the other hand, not all Cobalamin C and D deficiency can be identified by newborn screening using approaches based solely on elevated C3 [22]. Depending on the quality of data describing the confirmatory work-up, “MMA” cases without definite report of the final diagnosis might also include some cases with severe maternal vitamin B₁₂ deficiency, which can sometimes also be picked up by elevated C3 on newborn screening [23,24]. This aspect could result in a higher incidence for example using the 6 additional “undefined” MMA/PA/Cbl cases reported for Germany for the year 2004 (Table 2, last row, labeled with #), as these data in the national report did not undergo the same strict plausibility checks as for the national German screening panel from 2005 on.

Newborn screening data from Germany used to calculate the incidence of PA and MMA is currently limited to a pilot period of tandem-MS newborn screening in the years between 1998 and 2004, as the compulsory directive on the national screening panel implemented in 2005 does not include these conditions. There are however pilot studies currently ongoing in two screening centers in Germany (Munich and Heidelberg) including newborn screening for MMA, combined remethylation disorders and PA, now on-going which also use second-tier strategies [25,26]. First results from one of these pilot studies suggest an incidence of about 1/100,000 for each MMA and PA [25]. These pilot studies will provide more robust prospective data on PA and MMA incidence for the population in Germany over the following years.

Comparing the US results and the German results, the German results have better case definitions than the US results. This is probably due to the close relationship of the screening laboratory to the clinical practice and open communication of confirmatory testing and subsequently more precise reporting. Many of the US state newborn screening laboratories have not historically differentiated the remethylation defects (cobalamin C and D), PA and the MMAs (from MUT, Cobalamin A, and Cobalamin B) in their reports since they focused on the elevated propionylcarnitine marker. Several US laboratories are moving towards more specific reports with addition of ratios and other methods to prevent false positive rates.

Kuwaiti numbers were a surprise since a priori we expected higher numbers due to the population isolates on the peninsula and the high incidence of PA in the Saudi population [16]. In part this may be a consequence of the medical system (private clinics versus not private clinics) and that although universal screening is a goal, not all infants are screened. It may also reflect the population having children in Kuwait during the study. As a result, it is unknown whether these

results reflect population incidence or whether they are limited by ascertainment bias.

Identifying incidence in the local populations versus universal populations may help with more appropriate estimates for clinical studies and recruitment for these studies keeping in mind that higher than universal incidence would imply that the studied population is more likely to have common founder mutations or several limited founder mutations. Particular attention should be paid to these differing populations in that different responses to interventions may be seen.

This analysis did highlight that these disorders do occur with regularity in all three countries. This is important since several studies have demonstrated that adequate and aggressive treatment of these disorders is essential to benefit patients with these disorders. In addition the goal of NBS, early identification prior to damage in disorders which are seen in that population, can be accomplished [27,28].

Here, we have calculated the incidences of the branched chain intoxication-type disorders, maple syrup urine disease, propionic acidemia and methylmalonic aciduria in three countries. The incidences differ, probably not only due to ethnic and population differences, but also are impacted by the definitions each country (and/or region) uses to define a positive result (i.e. are only methylmalonic acidurias without homocystinuria included or do you include remethylation defects such as cobalamin C or D deficiency?). Thus, it is important when reporting incidence, to recognize the population tested and the definition used to define a positive test.

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